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THE UNIVERSITY OF ALBERTA

STRUCTURAL AND SYNTHETIC STUDIES ON LYCOPODIUM ALKALOIDS

by

© SARATU BINTA DIKKO

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA

SPRING, 1975

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "STRUCTURAL AND SYNTHETIC STUDIES ON LYCOPODIUM ALKALOIDS"

submitted by Saratu B. Dikko

in partial fulfilment of the requirements for the degree of Master of Science

W.A. Ayer, Supervisor.

K. Kopecky.

A. Locock,
External Examiner.

Date 5th Dec 1974

ABSTRACT

In cooperation with Dr. J.H. Wilce, a botanist interested in the taxonomy of the plant family Lycopodiaceae, we have undertaken an examination of the alkaloidal content of three species of Lycopodium indigenous to Columbia, South America.

Two of the three plants, L. thyoides and L. contiguum were studied in some detail.

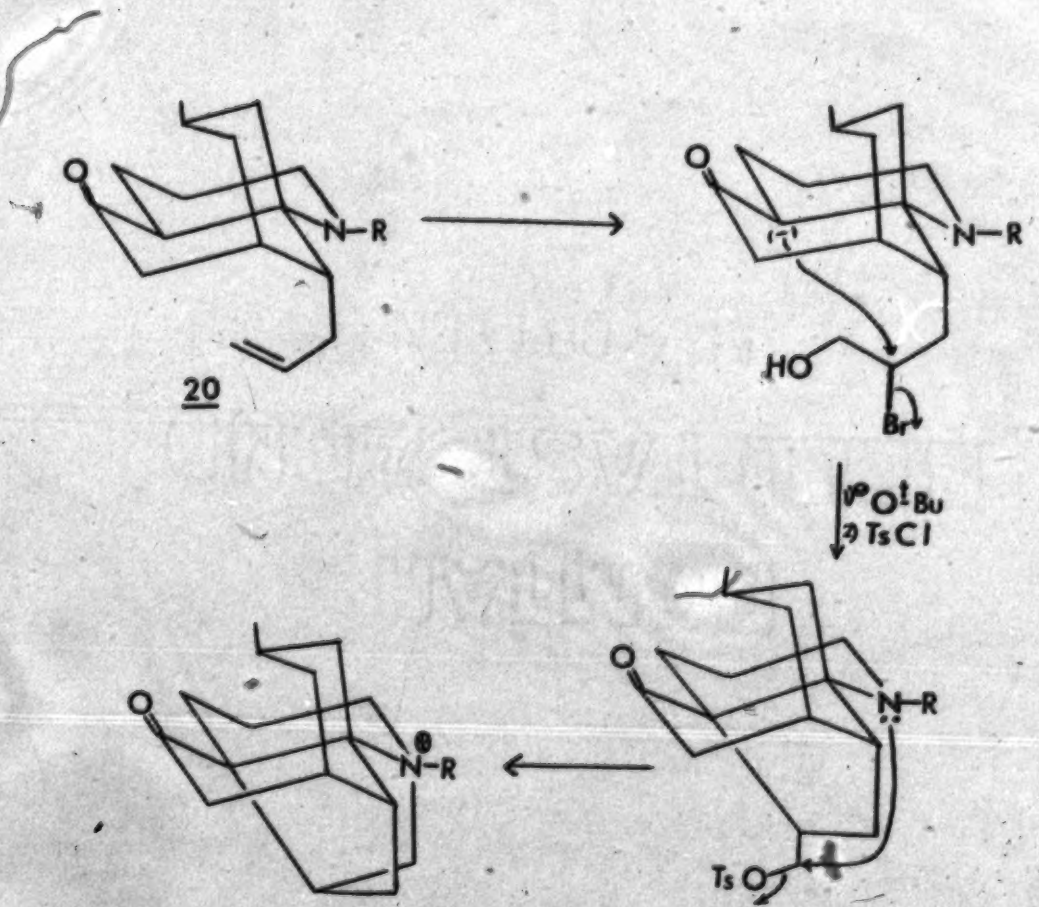
A total of five alkaloids were isolated from L. thyoides, four of these were positively identified as lycopodine 1, O-acetylfawcettiine 30, O-acetyldihydrolycopodine 31, and fawcettiine 32. The fifth compound isolated has not been identified. Five alkaloids were also isolated from L. contiguum. Again four of them were positively identified as lycopodine 1, O-acetylfawcettiine 30, clavolonine 35, and fawcettiine 32. The fifth compound is similar to that from L. thyoides and remains also unidentified.

Very small quantities of two alkaloids were isolated from the third plant, L. reflexum, and were shown to be related to α- and β-obscurines (35, 35a).

An attempt to convert the lycopodine skeleton 1 to the lycopecurine skeleton 14 has been investigated. The olefin 20 was the target intermediate, which might be transformable into the lycopecurine skeleton 14 as shown in scheme 3.

Both the Cope-elimination on lycopodine N-oxide and the Hofmann-elimination on dihydrolycopodine methiodide have been investigated in attempt to prepare the olefin 20. In neither case was it possible to obtain the desired intermediate. The products obtained in these reactions are discussed.

Scheme 3



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The National Research Council of Canada and the University of Alberta for financial assistance.

Dr. J.H. Wilce for providing plant material and also for classifying those plants.

The technical staff members of the Department of Chemistry, especially Mr. R.N. Swindlehurst and associates, Dr. T. Nakashima and associates, and Dr. A.M. Hogg and associates for the determination of various spectra.

Mrs. J. Ball for the graphic work.

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INTRODUCTION

Considerable work has been done on the alkaloids of the family Lycopodiaceae, and as mentioned below, ten different types of carbon-nitrogen skeletons have been encountered. No attempt appears to have been made to relate the alkaloidal content to taxonomical differences within the genus Lycopodium. It has been noted[1] that lycopodine-type alkaloids 1 are widely distributed within the genus, being absent only in L. cernuum. The latter species contains only alkaloids of type 5.

In 1970, we received a request from a taxonomist, Dr. Joan H. Wilce, University of Massachusetts, to identify the alkaloids present in several species of Lycopodium native to South America, as part of a program to clarify the taxonomy in the genus. In Dr. Wilce's words, "...before a taxonomist can evaluate the data from those species, he needs a broader picture than those species alone provide - at least in Lycopodium. That is the reason I have taken it upon myself to offer to collect material for chemical studies at the same time I collect for my own morphological work. I hope to see the chemical data from many more species one day!"

Our continuing interest in the alkaloids of Lycopodium prompted us to participate in this program. This would at the same time provide us with the potential for

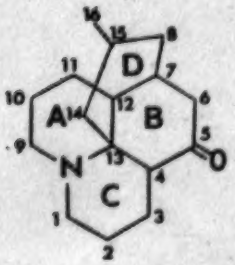
discovery of new alkaloids of possible biogenetic interest. Since alkaloids in general have proven to be a rich source of physiologically active compounds, this potential may also be explored.

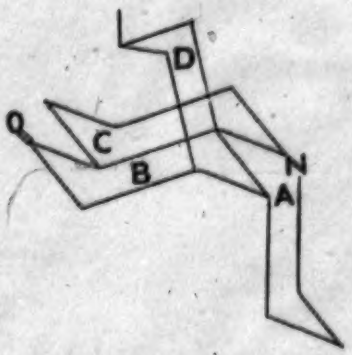
The Lycopodium alkaloids have a long history dating back to 1881 when Bödeker reported the isolation of a base from Lycopodium complanatum, most likely what we now know as lycopodine. However, nothing further was done about such bases, aside from reporting their existance in several other species, until Achmatowicz and Uzieblo in 1938 assigned the correct formula to lycopodine. They also isolated and characterized three other alkaloids from the Polish plant, Lycopodium clavatum.

In the 1940's, after examining various species, Manske and Marion reported that the Lycopodium family was a rich source of alkaloids. From that time on, the study of the chemistry of the Lycopodium alkaloids has been an area of active interest. Until the mid 1960's, studies were mainly concerned with isolation and structure determination. Since the mid 1960's, work has steadily advanced into the area of synthesis, and informative studies on the biosynthesis of these alkaloids has been carried out. Over one hundred alkaloids have been isolated from Lycopodium plants and comprehensive reviews of the work done up until 1973 have been provided by MacLean[1] and by Ayer.[2]

The alkaloids have been divided into ten groups,[2] based on their carbon-nitrogen skeleton system. These groups are listed below with an example from each group. The numbering system employed is adapted from that first suggested by Wiesner.[3]

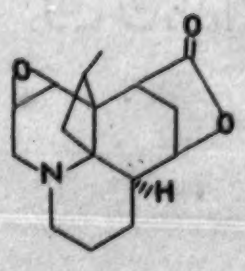
The LYCOPODINE group.





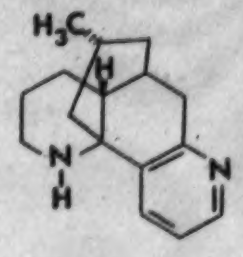
Lycopodine 1

The ANNOTININE group.



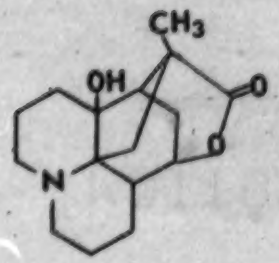
Annotinine 2

The LYCODINE group.



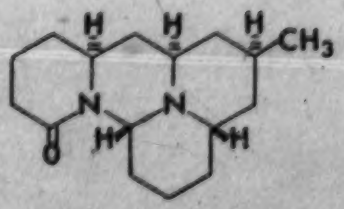
Lycodine 3

The ANNOTINE group.



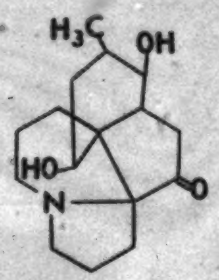
Annotine 4

The CERNUINE group.



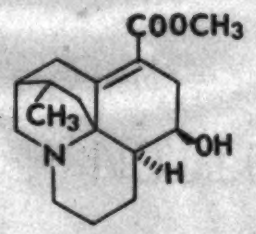
Cernuine 5

The SERRATININE group.



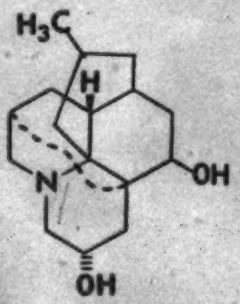
Serratinine 6

The ANNOPODINE group.



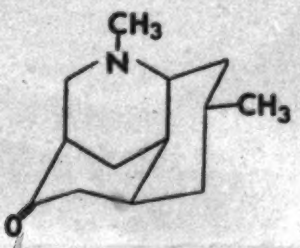
Annopodine 7

The ALOPECURINE group.



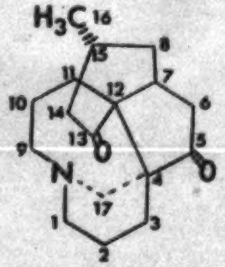
Debenzoylalopecurine 8

The LUCIDULINE group.



Luciduline 9

The LYCOFLEXINE group.



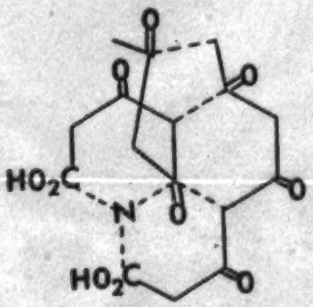
Lycoflexine 10

Of these groups, lycoflexine is the most recent addition, the structure of which was reported by Ayer and co-workers in 1973.[4] It is a C_{17} compound isolated from Lycopodium clavatum var. inflexum, whose structure was confirmed by x-ray crystallography.

At the time of writing of this thesis, only lycopodine 1,[5,6] annotinine 2,[7,8] and serratinine 6[9] had been prepared by total synthesis. Dihydrodeoxyepiallocernuine, a degradation product of cernuine, had also been prepared[10] and lycopodine has been transformed into lycodine thus furnishing a formal synthesis for lycodine.[11] These syntheses have been reviewed by Ayer.[2]

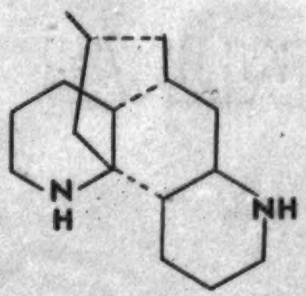
The Lycopodium alkaloids present an interesting biosynthetic problem. It was first believed that biosynthesis

of these alkaloids involved the condensation of two 8 carbon polyketide chain units and the appropriate nitrogen source.[12] The way in which these two chains condense is illustrated by the numbering system proposed by Wiesner.[3] If this were the case, then the lycopodine skeleton would have arisen as follows:



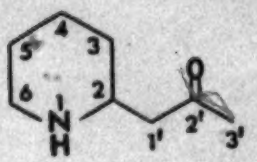
11

It was later recognized that the lycodine and cernuine skeletons could have arisen from the condensation of two 2-propylpiperidine units[13,14] as shown:



12

2-Propylpiperidine is a naturally occurring alkaloid (coniine), and had been shown to be derived via a polypeptide pathway.[15] In pelletierine 13, an alkaloid similar to coniine, only the side chain originates from acetate. The piperidine ring is derived from lysine.



pelletierine 13

Recent studies have been directed at determining which of these two pathways is actually followed in the biosynthesis of the Lycopodium alkaloids.

In the polyketide route, the odd-numbered carbon atoms represent the acetate carboxyl, and the even-numbered carbon atoms represent the acetate methyl groups. Feeding 1- ^{14}C-acetate to the plants would lead to lycopodine labelled at the odd-numbered carbon atoms, while feeding 2- ^{14}C-acetate would lead to lycopodine labelled at even-numbered carbon atoms.

Since the carboxyl group of the acetate gives rise to only C-2' of pelletierine, 1- ^{14}C-acetate by the pelletierine pathway would give lycopodine labelled only at C-7 and C-15, while 2- ^{14}C-acetate (i.e. C-1' and C-3' of pelletierine) would give lycopodine labelled at C-6, C-8, C-14 and C-16.

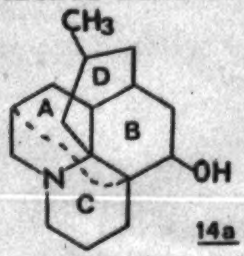
Experiments show that when 1- ^{14}C-acetate is fed to L. tristachyum, and the radioactive lycopodine degraded, C-5 is unlabelled while the acetic acid formed by Kuhn-Roth oxidation (C-15 and C-16) contained about one half the total activity.[14] 2- ^{14}C-acetate provided lycopodine in which about one quarter of the activity was present at

C-15 and C-16. Since the polyketide pathway requires that only one eighth of the activity be at C-15, C-16, regardless of whether 1- ^{14}C-acetate or 2- ^{14}C-acetate was used, these results seem to preclude the polyketide pathway. Other experiments designed to test the pelletierine pathway, showed that both 2- ^{14}C-lysine and 6- ^{14}C-lysine were incorporated into lycopodine with one quarter of the activity at C-5 and one quarter at C-9 in each case.[14,16]

More recent studies[17] have shown that although pelletierine is incorporated into lycopodine, (and other Lycopodium alkaloids) only one unit (i.e. C-9 to C-16) is incorporated. The other half of the molecule, C-1 to C-8, is not derived from pelletierine even though it is generated from lysine and acetate. Thus the biosynthetic origin of these alkaloids remains an area of study.

In the work described in this thesis, the alkaloidal content of three Lycopodium plants was investigated. Two of the three plants, L. contiguum and L. thyoides, collected near Bogota, Columbia have been investigated in some detail. The third plant, L. reflexum, also collected near Bogota, Columbia, contained very polar alkaloids which were not separable by usual chromatographic techniques. Attempts to precipitate salts of the bases also failed. Acetylation of the crude bases decreased the polarity of some of the bases but still no pure compounds could be isolated.

The later part of this thesis deals with attempts towards the synthesis of lycopecurine 14, an alkaloid belonging to the alopecurine group. Members of this group possess the pentacyclic skeleton exemplified by debenzoylalopecurine. This skeleton is the lycopodine skeleton with an additional bond between C-4 and C-10.



14a

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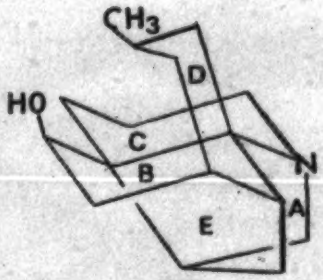
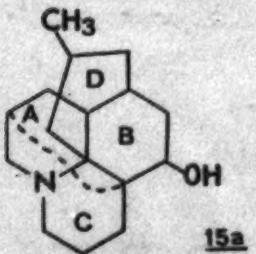
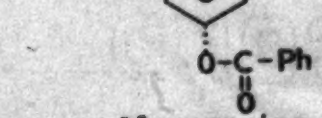


14b

Lycopecurine



15a



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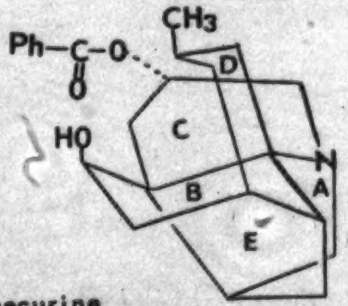


15b

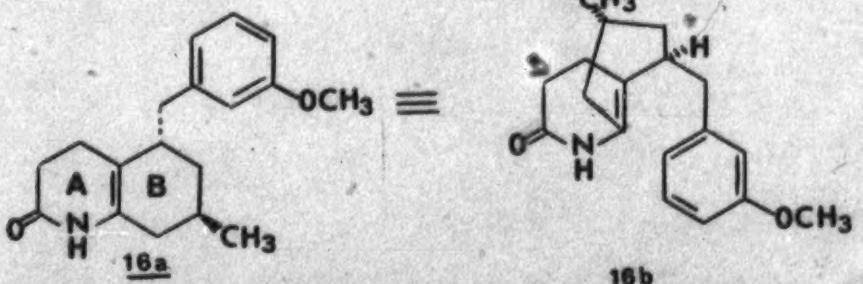
Alopecurine

Alopecurine 15, the only benzoylated Lycopodium alkaloid reported up to date, was the first member of this group of pentacyclic alkaloids to be isolated. It was isolated from L. alopecuroides and its structure determined by x-ray studies on the methobromide, and on debenzoylalopecurine hydrobromide. Like alopecurine, lycopecurine was isolated from L. alopecuroides and its structure determined by x-ray studies. It is interesting to note that in alopecurine, ring C is in a twist conformation, whereas

in lycopercurine it is in the chair form. The non-bonded interaction between the oxygen substituent at C-2 in alopecurine and the exo-hydrogen at C-9 is apparently sufficient to make the twist form the favoured conformation. In the absence of such interaction, the ring remains in the more usual chair form.

The alopecurine skeleton being basically that of lycopodine with one additional bond, we decided to investigate methods for transforming lycopodine 1 into lycopecurine 14. Since lycopodine has been synthesized,[5,6] a successful conversion of lycopodine into lycopecurine would furnish a total synthesis of lycopecurine.

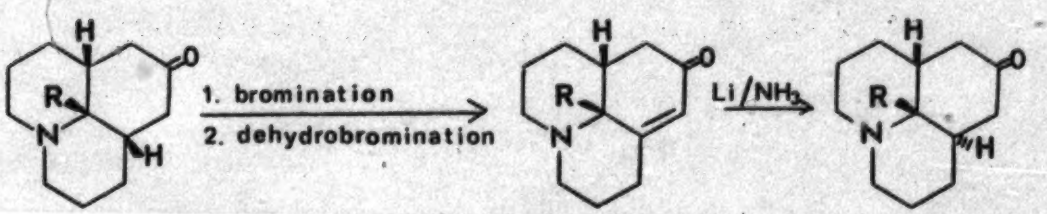
Prior to the first two successful syntheses of lycopodine,[5,6] 12-α-lycopodine, which is not a naturally occurring alkaloid, had been successfully synthesized.[18] The difficulty in establishing the natural configuration at C-12 was overcome in both syntheses of lycopodine itself. In one approach, the correct stereochemistry at C-12 was obtained by acid-catalyzed cyclization of the racemic hexahydroquinolone 16.



16a ≡ 16b

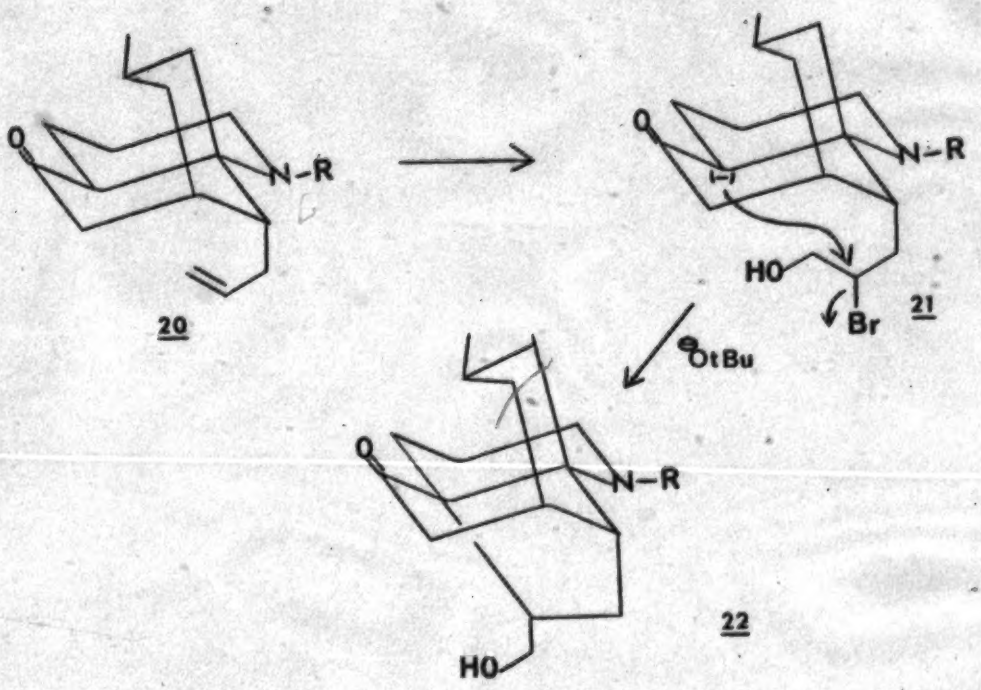
In the other approach, this problem was overcome by

the discovery that α,β-unsaturated ketones 18, are reduced by lithium-ammonia to give mainly the cis-trans ketones 19.



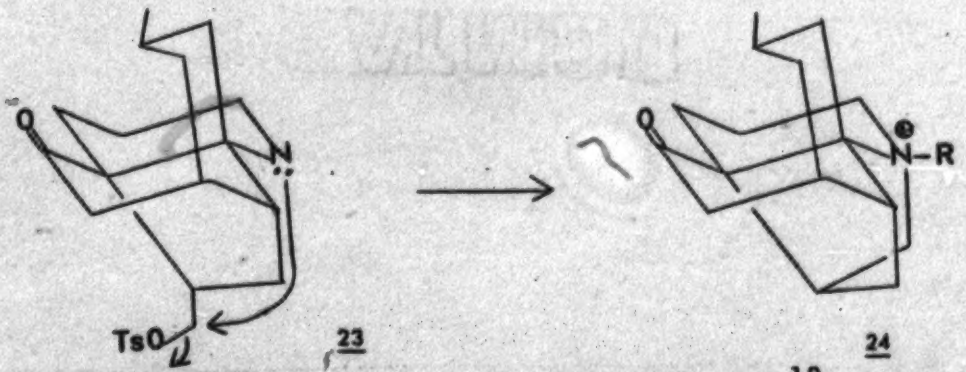
17 18 19

In our approach to the synthesis of lycopecurine, we intended to cleave ring A of lycopodine between N and C-9, to obtain an olefinic double bond between C-9 and C-10, 20, which could be bifunctionalized with a peracid followed by HBr. Treatment of the resulting compound 21 with a strong base should give an anion at C-4, which could then ring close by displacing the bromide ion from C-10 in an S_N2 fashion (21 to 22).



20 21 22

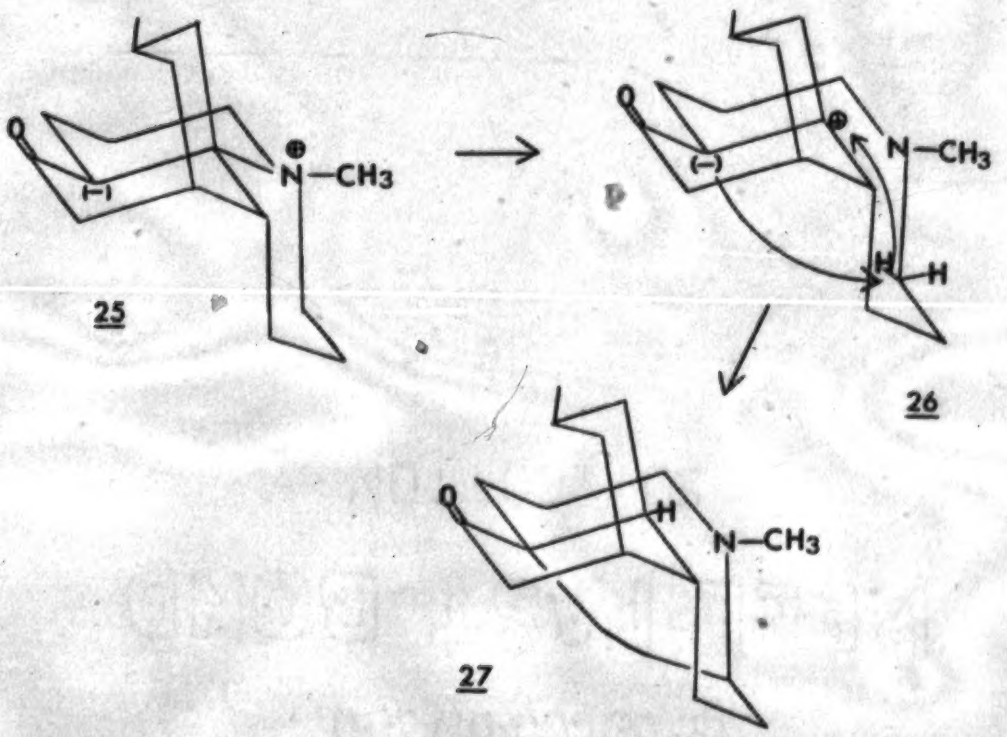
Conversion of the hydroxyl at C-9 to a better leaving group such as tosylate, followed by displacement of the tosyloxy group by the nitrogen lone pair would yield the desired pentacyclic system, (23 to 24).



23 24

Since lycopodine methiodide has been shown[19] to give a highly unusual rearrangement product 27 when treated with

strong bases such as potassium tert-butoxide, normal Hofmann elimination does not lead to the desired olefin, and other methods had to be investigated.



25 26 27

RESULTS AND DISCUSSION

Plant material representing six species of Lycopodium was obtained from Dr. J. Wilce, University of Massachusetts. The material was collected in Columbia, South America. The following is a copy of Dr. Wilce's notes accompanying the collection:

L. thyoides. *CUNDINAMARCA. Bogota. Monserrate, summit, along trail leading away from church. "grass-shrub paramo". Alt. 3100-3200 M. J.W. 45-70. June 6, 1970.*

[distinguished from other collections sent for chemical analysis by its flattened branchlets having an upper row of scale leaves as well as a lower row and 2 lateral rows. Strobili on peduncles. Obvious relationship with the group of L. complanatum and L. flabelliforme.]

L. jussiaei. *CUNDINAMARCA. Road from Bogota to Villavicencio, Km. 14 just before Chipaque. Collection stop #2. Alt. 3080 M. Found in low vegetation at top of and scrambling over road cut. At same locality, in open grass-shrub paramo, collected L. thyoides, the two "selago things" (coarse and fine), and L. contiguum. J.W. 37-70. June 7, 1970.*

[only other flat-branched species sent. lacks dorsal row of leaves seen in L. thyoides, is larger and coarser than L. thyoides. Strobili pedunculate.]

L. contiguum. *CUNDINAMARCA. Road from Bogota to Villavicencio. Stop #2 (see L. jussiaei above). Not seen on road cut, but abundant just beyond it. J.W. 41-70. June 7, 1970.*

[habit a bit like annotinum but leaves ascending, and more delicate than annotinum. Tips of leaves becoming scarious, giving a sheen to branchlets before tips are lost. Strobili sessile. Only collection sent without pedunculate strobili. -- no, cernuum too, but that's very distinct.]

L. clavatum. META. (probably, but need to check on detailed map as it was close to border of Cundinamarca). Road from Bogota to Villavicencio. Km. 104. Alt. 1170 M. Stop # 10. Abundant on roadside slopes, growing with cernuum and the "fine selago thing". J.W. 42-70. June 8, 1970.

[looks just like L. clavatum from North America.]

L. cernuum. META. Road to Bogota just outside Villavicencio, ca. Km. 109. Alt. 890 M. Collection stop # 9. Abundantly scrambling over roadside bank. J.W. 36-70. June 8, 1970.

[I do not know enough about this group to distinguish L. cernuum from what Herter lists as L. convolutum, found in Columbia along with cernuum, or from what he calls L. pendulinum from Peru, where cernuum is also listed. Some authors lump it all anyway. If your chemical data shows need for a name other than L. cernuum, let me know!]

L. sp. (the fine selago thing). CUNDINAMARCA. Road from Bogota to Villavicencio. Between Km. 65-70, on both sides of the river along the road and near bridge. Alt. 1250-1260 M. Collection stops # 5 and # 6 combined. J.W. 33-70 (stop 5) and J.W. 43-70 (stop 6). June 7, 1970.

The last mentioned species was later identified by Dr. Wilce as Lycopodium reflexum Lam. Voucher specimens are deposited in the University of Alberta Herbarium.

The initial extraction of the alkaloids was performed by Dr. H. Katayama of these laboratories and is described in the Experimental part of this thesis. Dr. Katayama showed that the alkaloids present in the South American L. cernuum were identical with those present in L. cernuum of Jamaican origin previously investigated in these laboratories.[20] He also showed that the L. clavatum alkaloids were identical with those of L. clavatum of Canadian origin. Similarly, the alkaloids of L. jussiaei were shown to be identical with those of L. annotinum collected in Canada.

However, he did not investigate the alkaloidal content of the remaining three plants viz. L. thyoides, L. contiguum, L. reflexum.

At the onset of the work described in this thesis, there was available only small quantities of alkaloidal material from these three species, and the thin-layer chromatograms of the crude basic extract of all these species showed that each was a complex mixture. The way in which the isolation and identification of the components was accomplished is discussed below. Each species will be discussed separately.

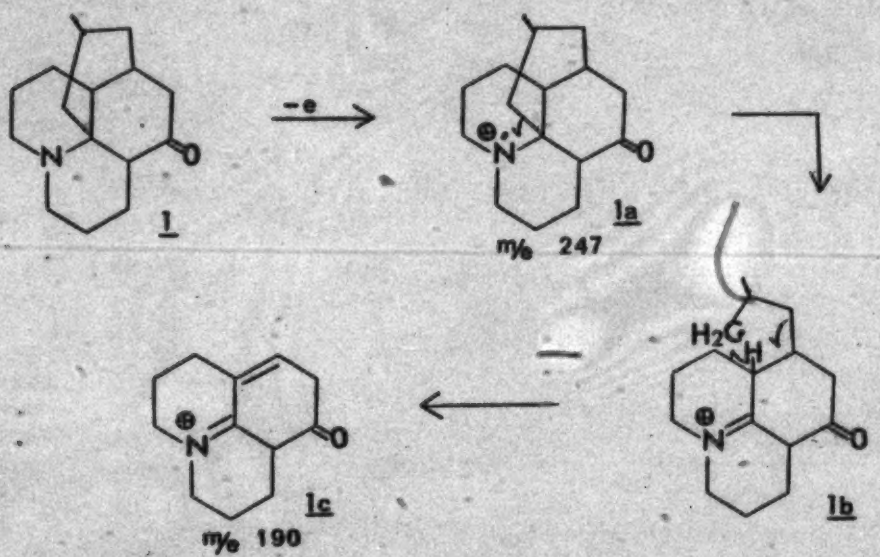
I. LYCOPODIUM THYOIDES

Isolation and Identification of Alkaloids

The best solvent system for separation of the alkaloidal components of this plant was found to be chloroform-methanol (49:1). In this solvent mixture, a thin layer chromatogram (tlc) of the crude alkaloids indicated at least five spots on aluminum oxide G (Figure 1) when developed with Dragendorf's reagent. These spots have been labelled A,B,C,D,E, beginning with the least polar, and they will be discussed in that order.

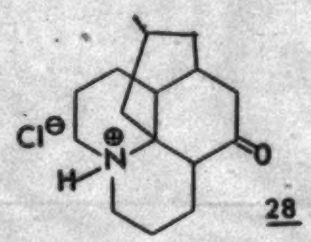
When the crude extract from L. thyoides was dissolved in acetone, a small quantity of a crystalline compound, P_1, separated on cooling. This crystalline compound P_1, was purified by recrystallization from methanol in an ether atmosphere. 11 mg of the recrystallized material was obtained. These crystals do not melt below 300°C. The infrared spectrum of P_1, Figure 3a, shows an $\overset{\oplus}{N}H$ absorption bond at 2460 cm^{-1}(broad), a carbonyl at 1705 cm^{-1} and active methylene at 1420 cm^{-1} ($-CH_2-\overset{O}{\overset{\|}{C}}-$), while the mass spectrum shows a molecular ion at m/e 247 and a most abundant fragment at m/e 190. Fragmentation from the molecular ion to the most abundant ion involves the loss of 57 mass units (247 to 190). This is a well known fragmentation for the lycopodine-type alkaloids. Since lycopodine itself is known to lose 57 mass units, the "bridging" carbons C-8, C-14, C-15 and C-16, from the molecular ion (m/e 247)

to the most abundant ion m/e 190, we decided to compare P_1



with an authentic sample of lycopodine. Although tlc (alumina) showed that they both have the same R_f value, lycopodine itself melts at 116°C[1] and it does not have an NH absorption band in its infrared spectrum. An alternative is that P_1 is a salt of lycopodine. If lycopodine was present in the crude alkaloidal extract from the plant material, it could have formed its hydrochloride salt during the process of extraction, by reaction with HCl liberated from the chloroform used in the extraction. Comparison of the infrared spectrum of P_1 with that of authentic lycopodine

hydrochloride showed that they were superimposable. Therefore, the crystalline compound P_1 was identified as lycopodine hydrochloride, 28.



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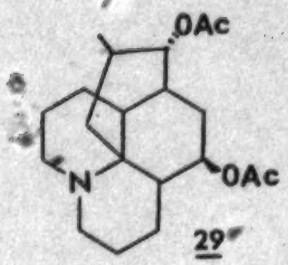
Since this is a salt, one would not have expected it to be so mobile on tlc. But this can be explained if one considers that the plates and columns were of basic alumina. Presumably, the free base is liberated during chromatography since later separations over basic alumina columns gave only the free base.

Elution chromatography of the acetone soluble crude basic extract over acid washed alumina (Shawinigan) was found to give better separation than aluminum oxide activity II. The solvent mixture for the separation was chloroform-methanol (49:1), with which four compounds were isolated. The fifth compound was eluted with chloroform-methanol (19:1). It is interesting to note that only about 66 % of the alkaloids could be recovered from the column even with very polar solvents ($CHCl_3$-MeOH, 9:1).

The first basic compound isolated, compound A, shows in its infrared spectrum (Figure 3), a carbonyl absorption

band at 1705 cm^{-1} and active methylene at 1420 cm^{-1}. Its mass spectrum shows a molecular ion at m/e 247 and a base peak at m/e 190, again characteristic of lycopodine. Comparison of the infrared spectrum of A with that of authentic lycopodine showed they were identical. Thus compound A was identified as lycopodine.

Compound B, which follows lycopodine off the column, was crystallized from ether, yielding 16 mg of crystalline material. It was purified by sublimation (110°C, 0.05 mm Hg.) as a colourless viscous liquid, which solidifies to a white solid (5 mg). The infrared spectrum of compound B, (Figure 4), shows an ester carbonyl absorption band at 1742 cm^{-1}, while its mass spectrum shows a molecular ion at m/e 349 with the most abundant fragment at m/e 234. Other significantly abundant fragments appear at m/e 289, 185, 174. The fragmentation pattern here indicates the loss of two acetic acid units, 60 mass units (349 to 289 and 234 to 174) which suggests the presence in the molecule of two O-acetyl groups. A loss of 55 mass units (289 to 234) suggests the possible loss of a lycopodine "bridge" that is deficient two hydrogen atoms. Such behaviour is consistent with two known lycopodine-type alkaloids, O-acetyllofoline 29, and O-acetylfawcettiine 30 (otherwise known as Lycopodium Base K).



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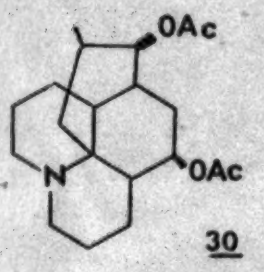

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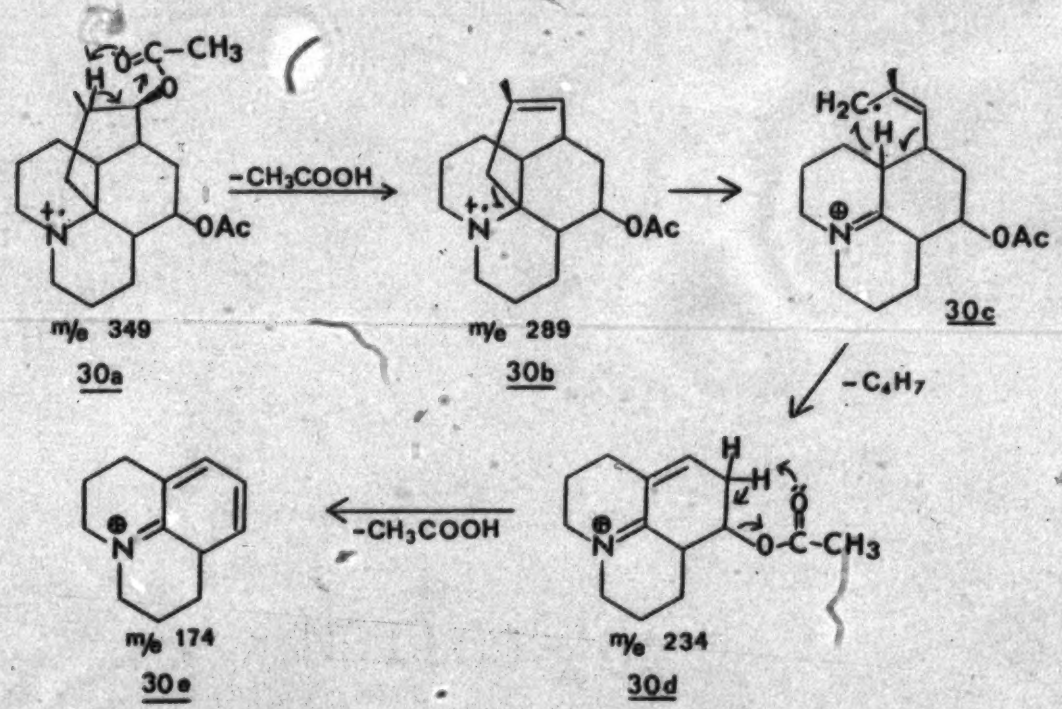
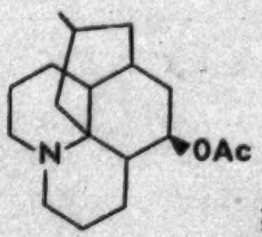
We then proceeded to compare compound B with each of these two alkaloids. Our source for O-acetyllofoline, was the so-called alkaloid L9 which is a 1:1 mixture of lycopodine and O-acetyllofoline. Alkaloid L9 crystallizes from hexane, m.p. 121-122°C but shows two spots on tlc,[21] the lower of the two being O-acetyllofoline and the upper, lycopodine. While the co-tlc of compound B with L9 showed three spots, only one spot was seen in the case of compound B with Base K, indicating that the two compounds have the same R_f value. For further comparison, 6 mg of the crystalline methiodide of compound B was prepared by treatment of the alkaloid with methyl iodide in acetone. These crystals melted at 271-272°. No melting point is reported for Base K methiodide, thus the methiodide of authentic free base was prepared and its m.p. also found to be 271-272°C. The mixed melting point of the compound B methiodide and Base K methiodide was 269-271°C. In addition, the infrared spectra (Figure 4a) of the two methiodides are

identical. We therefore concluded that compound B is O-acetylfawcettine (Base K) 30. The fragmentation pattern in the mass spectrum would be as shown:



Compound C, which has a lower R_f value than Base K, is the most abundant alkaloid (tlc) in this species. 24 mg of crystallized material were obtained from pentane-ether, m.p. 94-96°C. The infrared spectrum of compound C shows an ester carbonyl absorption band at 1732 cm^{-1} (Figure 5). Its mass spectrum shows an apparent molecular ion at m/e 291, the most abundant fragment at m/e 234 and a 174 peak. The mass spectrum shows a loss of 57 mass units (291 to 234), which has been encountered in the lycopodine

case (loss of the "bridge" carbon atoms), and a further loss of 60 mass units (234 to 174) as in the case of Base K (due to the loss of one molecule of acetic acid). This data coupled with the ester absorption in the infrared spectrum, strongly suggests a lycopodine-type alkaloid with one O-acetyl group which is not located on the "bridge". There is a known lycopodine-type alkaloid with a similar set of data, O-acetyldihydrolycopodine, 31.



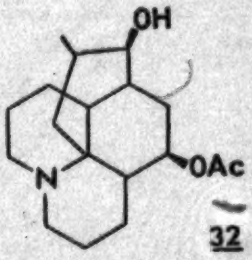
31

Compound C was compared with an authentic sample of O-acetyldihydrolycopodine. The two compounds showed the same R_f value on tlc (alumina). Six mg of the hydroperchlorate salt of compound C was prepared, and purified by crystallization from acetone in an ether atmosphere, m.p. 246-247°C (lit 246-247°C).[1] The infrared spectrum (Figure 5a) was compared with that of authentic O-acetyldihydrolycopodine hydroperchlorate. The two infrared spectra were identical. Compound C is thus O-acetyldihydrolycopodine 31.

Compound D, the next compound isolated, was not obtained crystalline. However, since only 8 mg were available, we attempted to identify compound D from the non-

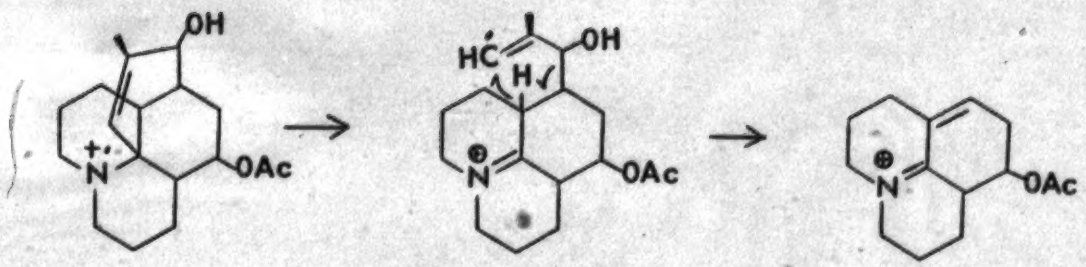
crystalline material. Its infrared spectrum (Figure 6), ($CHCl_3$) shows a carbonyl absorption band at 1720 cm^{-1}, and hydroxyl absorption at 3610 cm^{-1}. The mass spectrum, shows a molecular ion at m/e 307, a base peak at m/e 174 and significantly abundant fragments at m/e 234, and 146. Again we observe the m/e 234, and 174 fragments as in previous cases, and the loss of acetic acid (60 mass units) indicating the presence of an O-acetyl group not located on the "bridge". Unlike the previous compounds, compound D initially loses 73 mass units (307 to 234). Assuming that compound D is a lycopodine-type alkaloid and also the structure 30d for that fragment with mass 234, then the hydroxyl group indicated in the infrared spectrum (3610 cm^{-1}), would be on the "bridge". The loss of 73 mass units (307 to 234) indicates the loss of the "bridging" carbon atoms plus one hydroxyl group ($C_4H_9O \equiv 57 + 16$). Compound D was thus compared with a known lycopodine-type alkaloid with similar properties, viz. fawcettiine (β-lofoline) 32. Tlc comparisons showed that both compound D and fawcettiine have the same R_f value (co-tlc). Although the first attempt to make the hydroperchlorate salt of compound D failed to yield crystals, later attempts did give crystals of m.p. 272°C. Crystals of the methiodide were obtained from methylene chloride-methanol (49:1) m.p. 293-294°C (lit 296-297°C).[1] A comparison of the infrared spectra of compound D methiodide (Figure 6a), with that of authentic fawcettiine

methiodide showed they were identical. Compound D was thus proven to be fawcettiine 32.

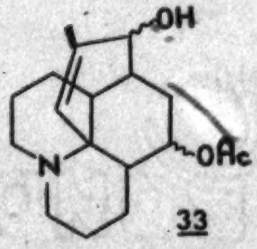


32

The final compound isolated, compound E, (eluted with chloroform-methanol (95:5)), was not obtained crystalline. However, there was obtained 2 mg of a crystalline hydroperchlorate m.p. 210-212°C. The infrared spectrum ($CHCl_3$) of the crystalline hydroperchlorate (Figure 7a) shows hydrogen bonded hydroxyl at 3350 cm^{-1}, a carbonyl at 1728 cm^{-1} and a 1230 cm^{-1} absorption band. While the infrared spectrum ($CHCl_3$) of the free base (Figure 7) shows free hydroxyl absorption at 3600 cm^{-1}, carbonyl absorption at 1735 cm^{-1} and olefinic absorption at 1650 cm^{-1}. The mass spectrum of the free base shows a molecular ion at m/e 305 ($C_{18}H_{27}O_3N$), base peak at m/e 174 and significant fragments at m/e 246, 234, 146. Assuming that compound E is a lycopodine-type alkaloid and structure 30d and 30e for the fragments m/e 234 and m/e 174 respectively, we can assign the loss of 71 mass units (305 to 234, C_4H_7O) to the loss of the "bridge" with a hydroxyl group (ir 3600 cm^{-1}) and an olefinic double bond (ir 1650 cm^{-1}).



Structures such as 33 and 34, are consistent with this data (the C-5 position of the O-acetyl group assigned only on biogenetic grounds).



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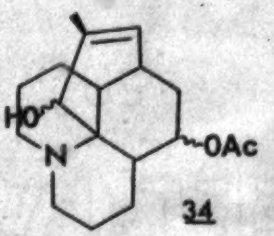


34

Insufficient material was available for n.m.r. and further study. Thus compound E has not been identified.

II. LYCOPODIUM CONTIGUUM

Isolation and Identification of Alkaloids

The tlc of the crude alkaloidal extract from this species of Lycopodium indicated at least five components (aluminum oxide G, methylene chloride - methanol, (49:1) developed with Dragendorf's reagent).

Isolation and identification techniques were similar to those employed in the structure elucidation of the alkaloids of L. thyoides.

The components of L. contiguum were isolated by column chromatography over aluminum oxide (BDH) using methylene chloride - methanol (49:1) as eluent. Four known compounds and one unknown compound were isolated.

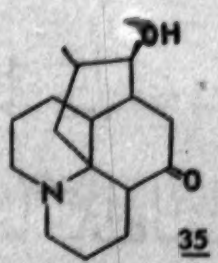
The components, which have been labelled F,G,H,I,J, Figure 2, in order of increasing polarity, were isolated and identified as described below.

The crystalline compound F (7 mg), which has a melting point of 115°C was identified as lycopodine 1, by comparison of tlc, ir, ms with an authentic sample. Also, the infrared spectrum of the hydroperchlorate salt of compound F, (mp 279-282°, lit[1] 283°) is superimposable on that of authentic lycopodine hydroperchlorate.

Compound G (7 mg) was obtained as a semi-solid, and identified as O-acetylfawcettiine (Base K) 30 by comparison of tlc, ir, and ms with an authentic sample. In addition, compound G, forms a crystalline methiodide

(m.p 271-272°) whose ir spectrum is identical with that of authentic O-acetylfawcettiine methiodide.

Compound H, (26 mg) the most abundant component (tlc), crystallizes from methyl acetate as colourless needles, m.p. 238°. The infrared spectrum (Figure 8) shows hydroxyl absorption at 3200 cm^{-1}, carbonyl absorption at 1685 cm^{-1} and an active methylene at 1410 cm^{-1}. The mass spectrum shows a molecular ion at m/e 263, the most abundant fragment at m/e 190 and other significant fragments at m/e 234, 174, 162. A loss in the mass spectrum of 73 mass units (263 to 190) could represent the loss of the "bridge" C_4H_9O, in a lycopodine-type compound, to give the tricyclic intermediate 1c, m/e 190. These data are consistent with a known alkaloid, clavolonine 35 (mw 263, m.p. 238°).



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A comparison of the infrared spectrum of compound H with that of authentic clavolonine, revealed their identity. In addition, the crystalline methiodide (methanol) of compound H, (m.p. > 300°) has an infrared spectrum (Figure 8a) which is identical with that of authentic clavolonine methiodide (m.p. 325-328°).[1] Compound H is therefore

clavolonine.

The most polar known component, compound I was isolated (15 mg) as an oil, then purified by sublimation (120°, 0.01 mm) to give a white powder. It forms a crystalline methiodide, m.p. 293-294°. Compound I was identified as fawcettiine (β-lofoline) 32, by comparison of tlc, ir, ms, with an authentic sample.

The most polar component isolated (9 mg), compound J, shows properties similar to those of the unknown compound isolated from L. thyoides. Its infrared spectrum shows carbonyl absorption at 1725 cm^{-1}, an olefinic double bond at 1650 cm^{-1} and a hydroxyl at 3600 cm^{-1}. The mass spectrum shows a molecular ion at m/e 305, the most abundant fragment at m/e 174 and other significant fragments at m/e 262, 246, 234, 146. Both compound E from L. thyoides and compound J from L. contiguum, have the same R_f value on aluminum oxide, and give just one spot when cochromatographed. Therefore, 33 and 34 are possible structures for compound J.

Below is a table summarizing the known alkaloids found in these two Lycopodium species, L. thyoides and L. contiguum, and their derivatives along with some literature data.

TABLE 1

Alkaloids of L. thyoides and L. contiguum

Alkaloid			m.p.°C	Derivatives & m.p.°C	Source
Mol. Wt.	Mol. Formula	Name	lit. (obs.)	lit. (obs.)	Source
247	$C_{16}H_{25}NO$	Lycopodine	116 (115)	Perchlorate 283 (279-282) Hydrochloride - (> 300)	L. thyoides L. contiguum
263	$C_{16}H_{25}NO_2$	Clavolonine	238 (238)	Methiodide 325-328 (> 300)	L. contiguum
291	$C_{18}H_{29}NO_2$	Acetyldihydro-lycopodine	95 - 96 (94 - 96)	Perchlorate 240-247 (246-247)	L. thyoides
305	$C_{18}H_{27}NO_3$	Unidentified	-	Perchlorate (210-212)	L. thyoides L. contiguum
307	$C_{18}H_{29}NO_3$	Fawcettiine (β-lofoline)	166 - 167	Methiodide 293-296 (293-294) Perchlorate 272-275 (272)	L. thyoides L. contiguum
349	$C_{20}H_{31}NO_4$	Acetylfawcettiine (Base X)	117	Methiodide (271-272)	L. thyoides L. contiguum

Since one of the objectives of this work was to suggest possible chemotaxonomical similarities between these species and others previously examined, Table 2 lists the various alkaloids isolated and the species from which each has been previously isolated. It may be seen that the alkaloids of L. thyoides most closely resemble those of L. clavatum and L. fawcettii. Those of L. contiguum, which indeed resemble those of L. thyoides except for the presence of clavolonine and absence of O-acetyldihydrolycopodine, also closely resemble those of L. clavatum, which contains both clavolonine and O-acetyldihydrolycopodine, and L. fawcettii. The taxonomic significance of these results, which have been communicated to Dr. Wilce, remains to be determined.

TABLE 2

List of Plants Containing Alkaloids Reported

Lycopodine	O-Acetyl-Fawcettiine	O-Acetyl-Dihydrolycopodine	Fawcettiine	Clavolonine
L. thyoides	L. fawcettii	L. clavatum	L. annotinum	L. alopecuroides
L. contiguum	L. thyoides	L. flabelliforme	L. clavatum	L. clavatum
L. annotinum	L. contiguum	L. thyoides	L. fawcettii	L. clavatum var. megastachyon
L. annotinum var. acrifolium			L. thyoides	L. densum
L. clavatum			L. contiguum	L. flabelliforme
L. clavatum var. megastachyon				L. contiguum
L. densum				
L. flabelliforme				
L. obscurum				
L. obscurum var. dendroideum				
L. tristachyum				
L. alopecuroides				
L. lucidulum				

TABLE 2 (continued)

	Lycopodine	O-acetyl-fawcettiine	O-acetyl-dihydrolycopodine	Fawcettiine
L. thyoides	√	√	√	√
L. clavatum	√		√	√
L. flabelliforme	√		√	
L. fawcettii		√		√

	Lycopodine	O-acetyl-fawcettiine	Clavolonine	Fawcettiine
L. contiguum	√	√	√	√
L. clavatum	√		√	√
L. fawcettii		√		√
L. annotiuum	√			√

III. LYCOPODIUM REFLEXUM

The alkaloidal content of this plant was examined by tlc on various types of adsorbants using several solvent systems. In most cases the material, which was very polar, did not move from the origin even in very polar solvents such as chloroform-methanol (9:1). In cases where some material moved, separation was very poor with a lot of streaking of material (Dragendorf). The various adsorbants and solvent systems investigated are listed in Table 3 below.

Since no separation could be achieved, the bases were re-isolated by acid-base extraction. The re-extracted bases behaved the same as the original extracts, on tlc.

In an attempt to decrease the polarity of some of the bases, the crude extract was acetylated (acetic anhydride, pyridine) and chromatographed over aluminum oxide, eluting with chloroform-methanol (49:1). Three reasonably clean (tlc) fractions were isolated (K,L,M), none of which was crystalline. Their properties will be discussed in order of increasing polarity.

Compound K, the least polar of the three compounds, shows a uv maximum (MeOH) at 305 nm. Its infrared spectrum shows carbonyl absorption at 1735 cm^{-1} and olefinic double bond at 1635 cm^{-1}. The mass spectrum shows a base peak at m/e 190 and other fragments at m/e 215, 347, 470, 527. It appears possible that this compound is a "dimeric"

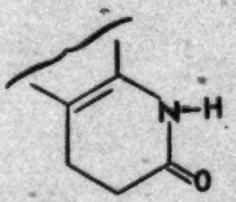
TABLE 3

Adsorbents and Solvent Systems for L. reflexum

Adsorbent	Solvent System												
		Chloroform-methanol					⬡:$CHCl_3$:Et_2NH			Ether-chloroform			
	$CHCl_3$	99:1	49:1	95:5	9:1	EtOAc	50:	40:	10	100:0	40:10	75:25	50:50	5:95
Aluminum Oxide G	✓	✓	✓	✓	✓	✓								
Magnesium Silicate				✓	✓			✓						
Silica gel				✓	✓			✓						
Polyamide	✓				✓									
Cellulose	✓			✓						✓	✓	✓	✓	✓

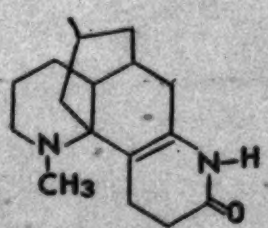
Lycopodium alkaloid, but further investigation was not possible due to lack of material.

Compound L shows a uv (MeOH) maximum at 250 nm. A characteristic absorption in this region is that due to chromophore (i), 255 nm, so compound L possibly contains such a chromophoric group.



(i)

The infrared spectrum of compound L shows N-H absorption at 3410 cm^{-1}, carbonyls at 1740 cm^{-1} (shoulder), and 1675 cm^{-1}, and possible olefinic double bond at 1630 cm^{-1}. In the mass spectrum the most abundant fragment appears at m/e 217 with other significant peaks at m/e 274, 231, 189. It was observed that compound L shows some properties characteristic of the known Lycopodium alkaloid, α-obscurine (m.w. 274) 35.

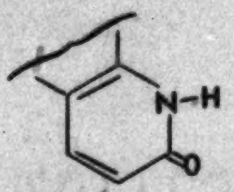


(35)

Tlc comparisons of compound L with authentic α-obscurine showed they had different R_f values (two spots on co-tlc).

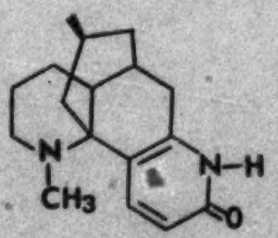
However, comparison with α-de-N-methylobscurine (tlc) showed they had the same R_f value. Attempts to sublime the free base, or to make either the hydroperchlorate or methiodide derivative of compound L all failed to yield a crystalline compound. Further studies could not be carried out since our supply of compound L was exhausted.

The most polar compound isolated, compound M, shows maxima in the uv (MeOH) at 312 nm and 230 nm. These maxima are characteristic of the chromophore (ii) which shows



(ii)

absorptions at 315 nm and 230 nm. The infrared spectrum of compound M shows bands at 1560 cm^{-1} and 1625 cm^{-1} and carbonyl absorption at 1665 cm^{-1} and 1745 cm^{-1} (shoulder). Its mass spectrum shows a base peak at m/e 215 and other peaks at m/e 272, 229, 187. Compound M was therefore compared with the known Lycopodium alkaloid that shows similar behavior, β-obscurine 35a.



(35a)

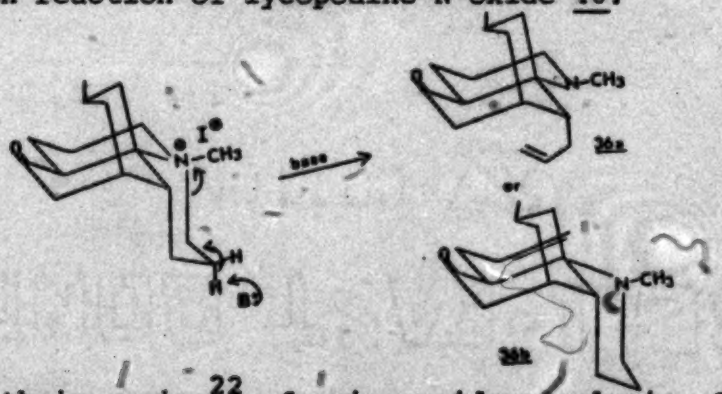
However, tlc comparisons show that compound M and β-obscurine have different R_f values. Comparison with β-des-N-methylobscurine (tlc) shows they have the same R_f value. Again sublimation attempts as well as attempts to make the methiodide or hydroperchlorate derivatives failed to give a crystalline compound. Again, lack of material precluded further investigation.

It is interesting to note the relationship between compound L and compound M, which occurs both in the uv and ms. Compound M seems to be a dehydro derivative of compound L, and they seem to be related to the α- and β-obscurines. Except for the mass spectral data, the compounds appear identical with the corresponding des-N-methyl compounds. It is possible that each compound is still impure, and that the mass spectra do not represent the major component but rather an impurity.

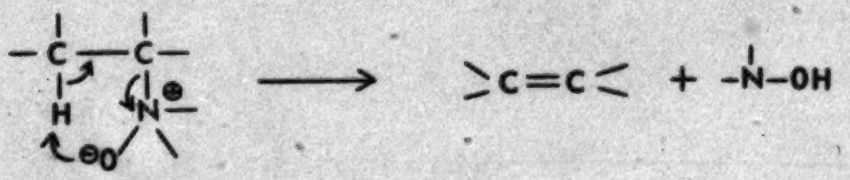
IV. TOWARDS THE SYNTHESIS OF LYCOPECURINE

In the attempted conversion of lycopodine 1 to lycopecurine 14, the immediate goal is the cleavage of ring A of lycopodine to give the olefin 36a.

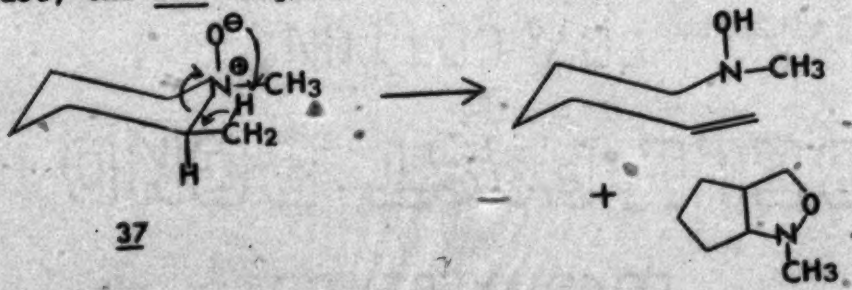
As mentioned previously, the Hofmann elimination reaction of lycopodine methiodide has been studied.[19] It was shown to give the rearrangement product 27, rather than the expected Hofmann products (36a and/or 36b). An alternative route to the olefin 36a could be through a Cope-elimination reaction of lycopodine N-oxide 40.



In their review[22] of amine oxide pyrolysis, Cope and Turnbull pointed out that tertiary amine oxides decompose when heated to give an olefin and a derivative of hydroxylamine. Although heating is generally required, the reaction may proceed at room temperature if DMSO is used as solvent (mixed with some water or THF for solubility) and the yields are high.[23] The reaction is believed to be first order and stereospecific in such a way that a planar five-membered cyclic transition state is required.

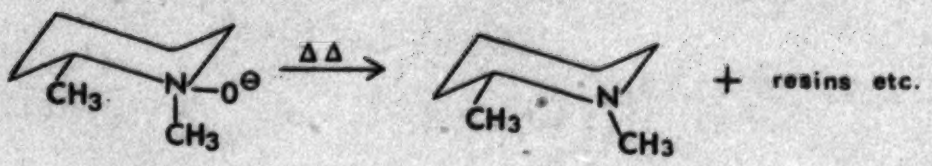


N-Methylpiperidine oxide does not undergo the Cope-elimination reaction.[24] However, the corresponding seven- and eight-membered ring compounds (N-methylperhydroazepine oxide and N-methylperhydroazocine oxide) in which the required transition state can form with less strain, give cleavage products in 53% and 79% yields respectively. While trans-N-methyl-α-pipecoline 37, gives some cleavage product, the cis compound does not react.



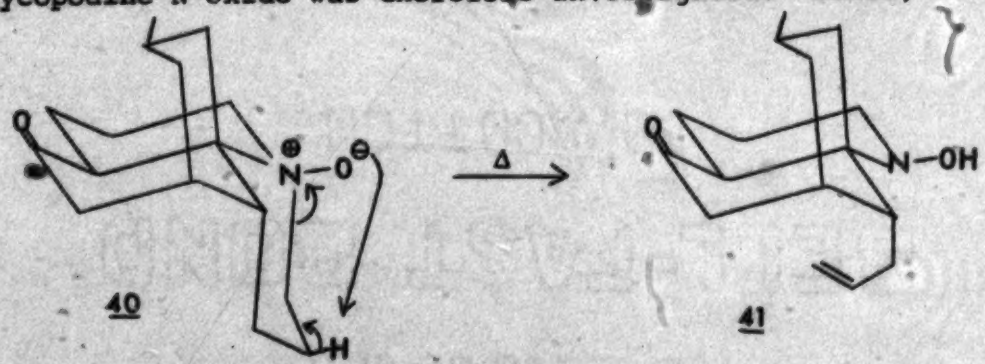
37

Activation of the β-hydrogen directs elimination towards the site of activation, otherwise elimination goes towards the β-position bearing the largest number of hydrogen atoms. When the Cope-elimination is interdicted by structure, amine oxides require higher temperatures for decomposition, as seen in the case of cis-N-Methyl-α-pipecoline 38.



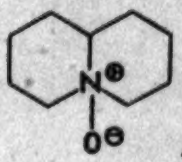
38 39

Examination of a molecular model of lycopodine N-oxide 40, reveals that the formation of a five-membered cyclic transition state is possible. Therefore, the N-oxide 40, could cleave via a Cope-elimination reaction to give the desired olefin 41. The Cope-elimination reaction of lycopodine N-oxide was therefore investigated. Since,

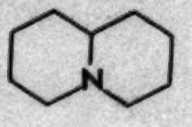


40 41

however, the quantity of lycopodine available was limited, we chose first to investigate the reaction using quinolizidine N-oxide 42 as a model compound. As with lycopodine N-oxide, quinolizidine N-oxide 42 can form the planar five-membered cyclic transition state required for elimination to occur.

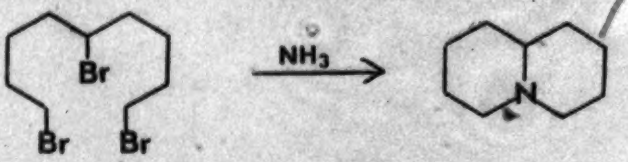


42



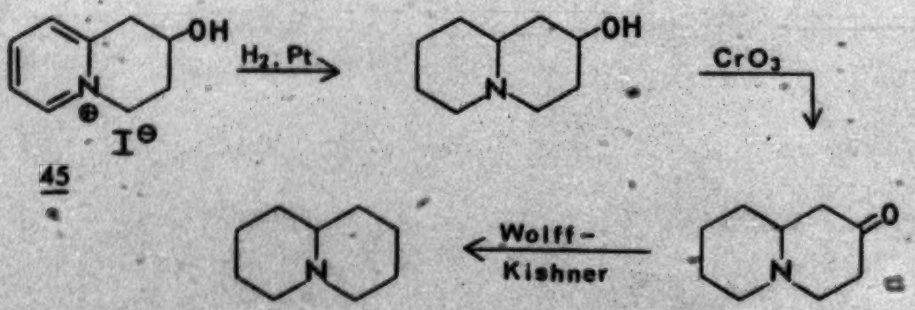
43

Quinolizidine 43, has been synthesized by two methods.[25,26] In one method,[26] Winterfield and Dunwald obtained quinolizidine by treating the bromo compound 44 with ammonia.



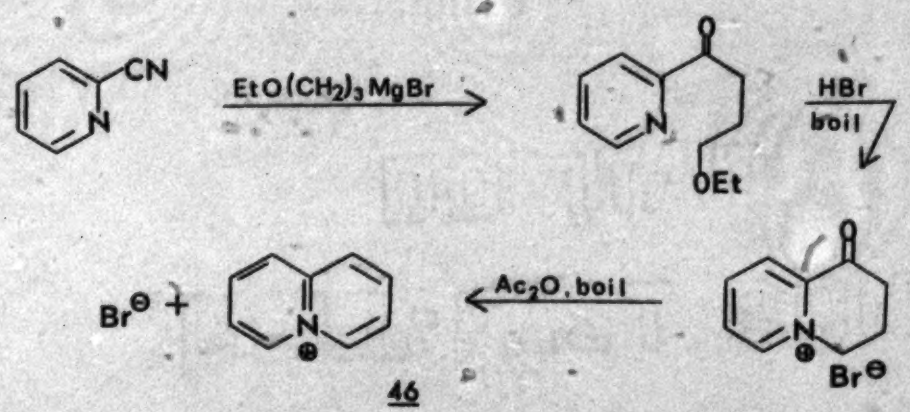
44

In the other method,[25] Boeklheide and Lodge employed a substituted tetrahydroquinolizidium salt 45 as a starting material. Hydrogenation followed by oxidation, then Wolff-Kishner reduction gave quinolizidine as shown below.



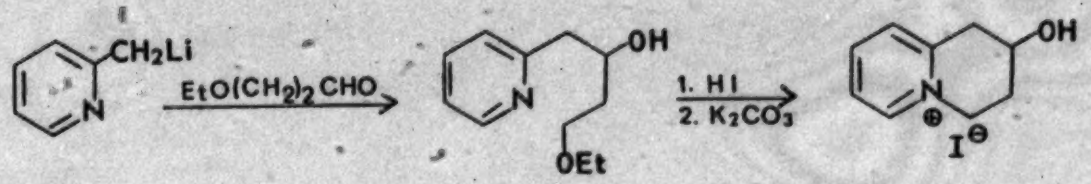
45

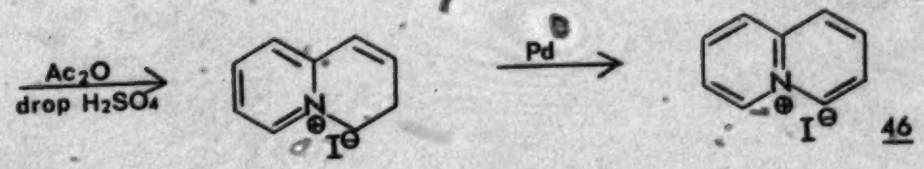
The quinolizinium ion 46 has been synthesized by various methods,[27-29] starting with α-substituted pyridine compounds. In their method Boeklheide and Gall[27] employed α-cyanopyridine as starting material. They treated it first with 3-ethoxypropylmagnesium bromide, then with hydrobromic acid. The resulting bromide ring closes immediately giving the corresponding bromide salt. Quinolizinium bromide 46 was obtained in 48% yield by treatment of the bromide salt with acetic anhydride (scheme 1, below).



46

Glover and Jones[28] improved on this. They treated a β-ethoxyaldehyde with picolyllithium, then substituted an iodine atom for the ethoxy group. The resulting iodide immediately ring-closes to give the corresponding tetrahydroquinolizinium iodide. Dehydration using acetic anhydride containing one drop of concentrated sulfuric acid, followed by dehydrogenation over palladium afforded the quinolizinium ion 46 in 96% yield (scheme 2, below).

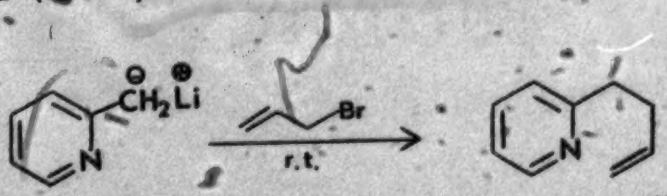




46

Catalytic hydrogenation of the quinolizinium ion 46 over platinum oxide afforded the fully saturated quinolizidine, as shown by Richards and Stevens.[29]

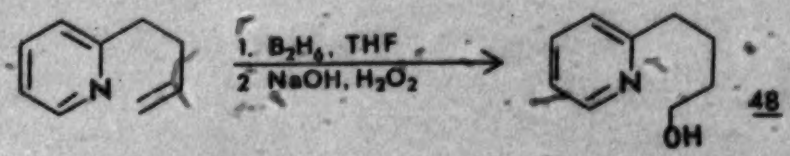
We prepared quinolizidine by a somewhat different route. α-Picolyllithium (from reaction of α-picoline with phenyllithium) was treated with allyl bromide to give a 53% yield of 2-(3-butenyl)pyridine 47. The colourless liquid so obtained (32.5°, 0.4 mm) shows absorptions due to



47

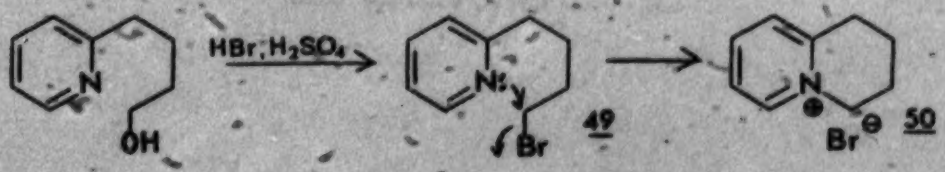
a monosubstituted double bond in its infrared spectrum at 1600 cm^{-1}, 985 cm^{-1} and 900 cm^{-1}. This data is corroborated by the appearance of multiplets at δ 5.0 and δ 5.85 in the n.m.r. spectrum.

Hydroboration of 47 gave 2-(4-hydroxylbutyl)pyridine 48 in 79.5% yield. The alcohol 48 distills as a colourless liquid (102°, 0.4 mm). Its infrared spectrum does not show olefinic absorption but does show hydrogen bonded hydroxyl absorption at 3100 - 3600 cm^{-1}. The n.m.r. spectrum (see experimental) is in full agreement with structure 48.



48

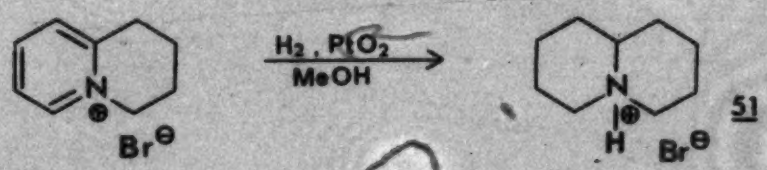
Ring closure was achieved by treatment of 48 with HBr containing a catalytic amount of concentrated H_2SO_4. Although the initial reaction product is 2-(4-bromobutyl)pyridine 49, the bromine atom is displaced by the nitrogen lone pair to give the bicyclic salt, tetrahydroquinolizinum bromide 50, as a crystalline solid (m.p. 237-240°) in 83.7% yield.



49 50

The infrared spectrum of 50 does not show hydroxyl absorption and the n.m.r. is consistent with the structure. Catalytic hydrogenation of 50 over platinum oxide gave the saturated compound, isolated as the crystalline hydrobromide

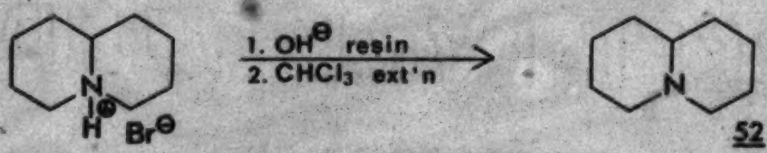
salt 51 (m.p. 292-293°) in 92% yield. Compound 51 analyses correctly for $C_9H_{18}NBr$. Its infrared spectrum shows $\overset{\oplus}{N}H$



51

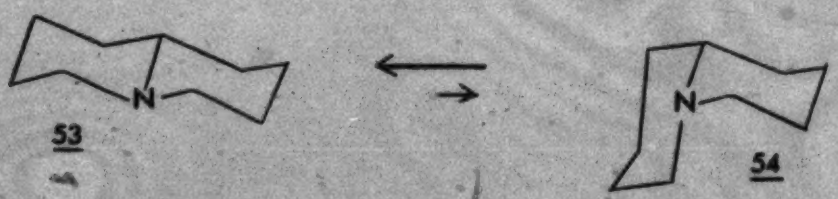
absorption at 3400 cm^{-1} and 2500 - 2700 cm^{-1}.

In order to liberate the free base from the salt formed, an aqueous solution of the salt was passed through an hydroxide-loaded ion-exchange column. Extraction of the aqueous solution with chloroform yielded quinolizidine 43. The infrared spectrum of quinolizidine 43 shows Bohlman



52

bands at 2670, 2740, 2780 cm^{-1}. This indicates that compound 43 exists predominantly in the trans conformation 53. The mass spectrum shows a molecular ion corresponding

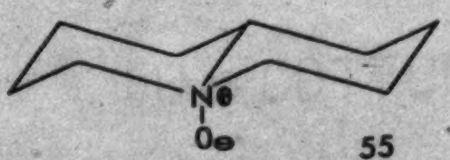


53 54

to $C_9H_{17}N$ (m/e 139.1355).

Quinolizidine N-oxide 42 was prepared by the method of Craig and Purushothaman.[30] In this method quinolizidine was treated with 99% meta-chloroperbenzoic acid[31] and the N-oxide purified by chromatography. The pure quinolizidine N-oxide was obtained in 80% yield as an oil. On standing at room temperature for a few days needle-like crystals formed within the oil.

The infrared spectrum of the N-oxide shows an intense $N^{\oplus}$-$O^{\ominus}$ absorption band at 950 cm^{-1} and its mass spectrum shows a molecular ion corresponding to $C_9H_{17}NO$ (m/e 155.1306). The ^{13}C n.m.r. spectrum shows only five signals indicating that the molecule is symmetrical. This is consistent with trans-quinolizidine N-oxide 55 as the ^{13}C spectrum of the cis compound would be expected to give nine signals.



55

Attempts to pyrolyse trans quinolizidine N-oxide did not give rise to a Cope-elimination product. The N-oxide was recovered (identified by i.r., m.s., tlc) as a colourless oil.

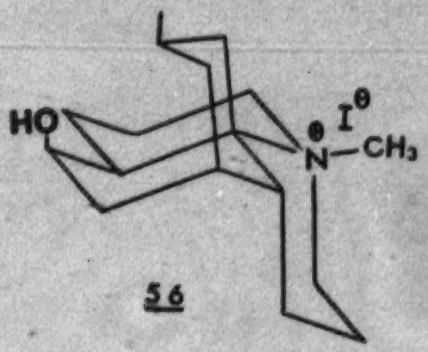
Lycopodine N-oxide 40 was prepared in a similar manner and obtained crystalline (m.p. 234-236°) from

pentane-ether in 81.2% yield. Its infrared spectrum shows a strong $\overset{\oplus}{N} - \overset{\ominus}{O}$ absorption band at 950 cm^{-1} and a carbonyl at 1710 cm^{-1}. The mass spectrum shows a molecular ion at m/e 263.1896 ($C_{16}H_{25}NO_2$).

Lycopodine N-oxide was subjected to the conditions of the Cope-elimination reaction. Heating under reduced pressures gave lycopodine (identified by i.r., m.s., tlc), while heating under reduced pressures in an inert atmosphere (N_2) gave lycopodine and sublimed lycopodine N-oxide.

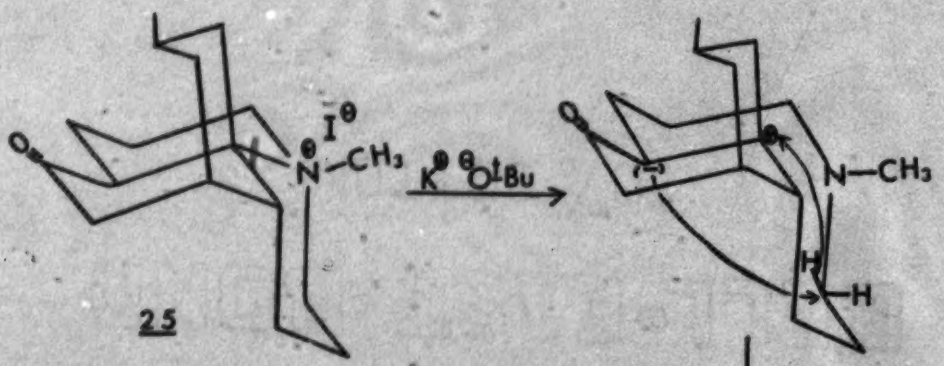
Since the Cope-elimination did not occur, we investigated the possibility of a base-catalysed cleavage reaction. Lycopodine N-oxide was heated with potassium hydroxide in freshly distilled ethylene glycol in an inert atmosphere (N_2). The reaction was followed by tlc which showed the formation of lycopodine from lycopodine N-oxide. The reaction was repeated in DMSO and under similar conditions lycopodine was again the only product. Also, it was shown that lycopodine is the product obtained when lycopodine N-oxide is heated alone in distilled ethylene glycol under nitrogen. It seems, therefore, that lycopodine N-oxide is heat labile and further investigations with this compound were discontinued.

Although lycopodine methiodide 25 has been shown to give an unusual Hofmann-elimination product 27,[19] there has been no report on the Hofmann product of dihydrolycopodine methiodide 56. It has been suggested that the

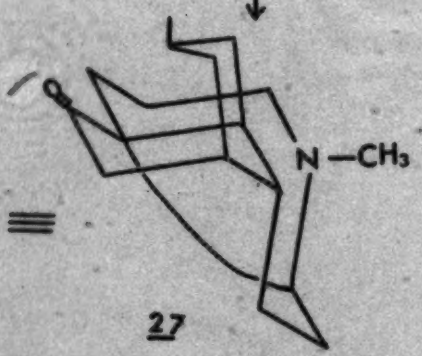


56

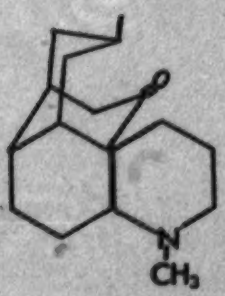
unusual product obtained from lycopodine methiodide results from abstraction of a proton α - to the carbonyl, followed by a molecular reorganization.[19]



25



27



Since the presence of the carbonyl serves to activate the α - hydrogens, we expected to remove this reactivity by reducing the ketone to the alcohol. Thus dihydro-

lycopodine methiodide 56, should undergo a normal Hofmann reaction rather than the molecular reorganization. Dihydrolycopodine methiodide was therefore prepared and its behavior with base investigated.

Dihydrolycopodine methiodide 56, prepared by treatment of dihydrolycopodine with methyl iodide, was allowed to react with potassium tert-butoxide in anhydrous ^{t}BuOH. The ether extract of the reaction product showed a large proportion (tlc) of very polar material along with two less polar components. The two nonpolar components were isolated. The least polar component was nonbasic and was not further investigated. The other nonpolar component was isolated as a low melting solid (0.020 g. from 0.20 g. starting material). Its infrared spectrum ($CHCl_3$, Figure 9) shows $>N-CH_3$ absorption at 2780 cm^{-1}, carbonyl at 1680 cm^{-1} and active methylene at 1405 cm^{-1}. The mass spectrum shows a molecular ion at m/e 261 and a base peak at m/e 57. Neither the i.r. nor the m.s. show properties consistent with the presence of the expected olefin 36a or 36b (mw 263). However, lycopodine methiodide has been shown[19,32] to give the same compound when treated with potassium tert-butoxide in ^{t}BuOH. Although the structure of the product of Hofmann-elimination of lycopodine methiodide was at first wrongly assigned,[32] it was later[19] shown to have the structure 27. Apparently, some of the dihydrolycopodine methiodide undergoes aerial oxidation during

the KO^{t}Bu - ^{t}BuOH treatment, giving rise to lycopodine methiodide. When the Hofmann reaction on dihydrolycopodine methiodide was attempted in anhydrous DMSO containing potassium tert-butoxide, a small amount of basic material was isolated. The reaction product isolated was mainly one compound. Its mass spectrum shows a base peak at m/e 58, and other intense peaks at m/e 263, 259, 244, 230, 216, 202, 187.

The reaction in DMSO containing freshly sublimed potassium tert-butoxide was repeated using an inert atmosphere (N_2). The total crude basic product obtained when the reaction was carried out at 80° was about twice that obtained when the reaction was carried out at room temperature. The major component, Q, isolated from the reaction product by preparative layer chromatography has, in both cases, the same properties. It does not show uv absorption above 220 mm. This indicates the probable absence of a conjugated diene. Its infrared spectrum ($CHCl_3$, Figure 10) shows $\geq$N-CH_3 absorption at 2780 cm^{-1} and olefinic absorptions at 3060 cm^{-1}, 1585 cm^{-1}, 975 cm^{-1} and 960 cm^{-1}. The mass spectrum shows an apparent molecular ion at m/e 273 ($C_{19}H_{31}N$) with a base peak at m/e 58. Other intense ions occur at m/e 258, 244, 230, 216, and 150. The exact masses of these fragment ions (see Experimental) all show the presence of nitrogen. The n.m.r. spectrum of compound Q, Figure 11, shows olefinic protons

~ δ 6.3, 5.7, 5.1, an N-methyl group at ~ δ 2.1 and a C-methyl group at ~ δ 0.9.

Compound Q forms a methiodide which crystallizes from ethyl acetate-methanol (2:1) in an ether atmosphere (m.p. 190-191°). The infrared spectrum of the methiodide, Figure 10a, shows apparent olefinic absorptions at 1590, 970, 985 cm^{-1}. Its n.m.r., Figure 11a, shows olefinic protons ~ δ 6.3, 5.7, 5.1, two $N-CH_3$ at ~ δ 3.25, 3.3, and $C-CH_3$ at ~ δ 0.95.

The structure of compound Q remains obscure. The apparent molecular formula $C_{19}H_{31}N$, indicates the addition of two carbon atoms during the reaction with the elimination of the oxygen function of dihydrolycopodine. The olefinic region of the n.m.r. spectrum may be interpreted in terms of the presence of vinyl group ($-CH=CH_2$) not further coupled, a methylene group ($>C=CH_2$) and a trisubstituted double bond ($>C=C<^{H}$) the hydrogen of which (δ 5.7) is further coupled. At this stage, we are unable to suggest a structure which satisfactorily accounts for the observation and further work will be necessary to explain this unusual reaction.

It thus appears that the Hofmann elimination on dihydrolycopodine, like that on lycopodine, does not proceed in a straight forward fashion and other approaches to the synthesis of the pentacyclic ring system of lycopecurine will have to be investigated.

EXPERIMENTAL

I. GENERAL

R_f value = distance moved by compound/distance moved by solvent.

Organic solutions were dried over anhydrous $MgSO_4$ before evaporation of solvent.

Melting points were determined on a Fischer-Johns hot-stage or Gallenkamp heating block melting point apparatus and are uncorrected.

Microanalysis was performed by the Microanalytical Laboratory of this department.

Infrared spectra were recorded on a Unicam SP 1000 grating infrared spectrometer or a Perkin-Elmer Model 421 dual grating spectrometer.

All infrared spectra of solids were determined in nujol, and all liquids on thin films unless otherwise specified.

Ultraviolet spectra were measured in spectral grade methanol using a Perkin-Elmer Ultraviolet Spectrophotometer Model 202.

Nuclear magnetic resonance spectra were measured in deuteriochloroform, unless otherwise specified, using a Varian Associates Model A-60 spectrometer or a Varian Associates Model HA-100 spectrometer with tetramethylsilane as internal standard.

^{13}C n.m.r. spectra were recorded on a Bruker HFX-90

spectrometer.

Mass spectra were recorded on an A.E.I. Model MS-9 mass spectrometer or an A.E.I. Model MS-2 mass spectrometer. Fragment ions are given as a percentage (in brackets) of the most abundant ion.

Chemical ionization mass spectra were recorded on an A.E.I. Model MS-12 mass spectrometer.

Thin-layer chromatograms were visualized with Dragendorf's reagent unless otherwise specified.

DRAGENDORF'S REAGENT (specific spray for nitrogenous bases).

Dragendorf's reagent consists of a mixture of two solutions A and B prepared as follows:

Solution A = 1.7 g. of basic bismuth nitrate [$Bi(NO_3)_3$] in 100 ml water|acetic acid (80/20).

Solution B = 40 g. of potassium iodide in 100 ml of water.

Mixture = 30 ml A|7.5 ml B|70 ml water.

THIN-LAYER CHROMATOGRAPHY

Two adsorbents were used throughout this work: silica gel G containing 1% electronic phosphor ($ZnSiO_4$, General Electric), and aluminum oxide G also containing 1% electronic phosphor. Slurries were made of 20 g. adsorbent in 40 ml water, and spread thinly (0.05 cm) and

evenly over clean, dry glass plates. The plates are allowed to air dry at room temperature for ~ 1 hr., then heated at 110°C in an oven for ~ 1 hr. They are allowed to cool and then stored in a dessicator until required.

PREPARATIVE THIN-LAYER CHROMATOGRAMS

The plates were prepared as described above for the analytical t.l.c. The components were visualized and marked by viewing under ultraviolet light (254 nm). A small strip of the plate was also sprayed with Dragendorf's reagent. The uv active and Dragendorf active zones were scraped off and eluted with appropriate solvents.

ELUTION CHROMATOGRAPHY

The adsorbent used in this work was aluminum oxide. The column was wet-packed with a non-polar solvent using the ratio, alumina to components, of 40 to 1. Excess solvent was removed from the column and the components to be chromatographed, dissolved in minimum amount of non-polar solvent, were applied to the column. The column was then eluted with the appropriate solvent.

II. ISOLATION AND IDENTIFICATION

Isolation of Alkaloids.

This work was done by Dr. H. Katayama of these laboratories.

The powdered Lycopodium plant material was extracted with methanol by using the Soxhlet continuous extraction

method. Evaporation of most of the methanol was followed by addition of dilute hydrochloric acid. The resulting slurry was filtered and the filtrate washed three times with ether, then basified with aqueous ammonia. The basic solution was then extracted with chloroform, dried and the solvent evaporated. The residue constitutes the so-called crude alkaloid of the plant. The ether extracts contain neutral and acidic compounds of the plant.

The following is a summary of the plants extracted and the alkaloidal content obtained.

Plant	Wt. of Dried Plant (g)	Wt. of Alkaloids (g)	% Yield by Weight
L. thyoides	1982	3.06	0.15
	2039	4.5	0.23
L. contiguum	963	1.682	0.175
	144	0.043	0.03
L. reflexum	1217	2.72	0.22

Preparation of Hydroperchlorates.

The base was dissolved in acetone, and $HClO_4$ in acetone added dropwise until the mixture was just acid to indicator paper pH 5-6. The solvent was then allowed to evaporate to dryness at room temperature. The residue was dissolved in a few drops of acetone and placed in an ether atmosphere. The crystals formed were collected by vacuum

filtration.

Preparation of Methiodides.

The base was dissolved in methanol (or acetone), a few drops of methyl iodide added and the mixture heated to reflux on a water bath for 10-20 min. In the cases where crystals formed they were collected by vacuum filtration. In those cases where crystals did not precipitate the solution was concentrated and placed in an ether atmosphere. The crystals thus formed were collected by vacuum filtration.

Acetylation of Bases.

The bases were dissolved in pyridine, a few drops of acetic anhydride added (~ 2 mg/drop acetic anhydride/2 ml pyridine) and the mixture allowed to stand at room temperature overnight. The solvent was evaporated and the acetylated bases separated by chromatography.

L. Thyoides.

A solution of the crude alkaloids (0.483 g.) in acetone upon cooling produced a crystalline material. These crystals were dissolved in a minimum amount of methanol and the solution was placed in an ether atmosphere. One large crystal formed (0.011 g.) m.p. > 300°. It was identified as lycopodine hydrochloride by comparison of its infrared (Figure 3a) spectrum with that of an authentic sample.

i.r.: (Figure 3a).

m.s. m/e: 247 (M^+10), 190(100), 162(9).

The crude alkaloid (0.462 g.) was placed on a column of aluminum oxide G (~ 20 g., activity II, 23 mm diameter) and eluted in 30, 5 ml fractions. Fractions 1 through 17 were eluted with chloroform-methanol (49:1, 85 ml), while fractions 18 through 30 were eluted with chloroform-methanol (19:1, 60 ml), fractions 1 and 2 contained only solvent. Fractions 3, 4, and 5 contained a non-basic material (0.006 g.) which was not further investigated. Fraction 6 showed two spots on tlc, one of which was the non-basic material also present in 3, 4, and 5; the other was a basic (Dragendorf positive) material. Separation of these two components was not attempted at this time. Fractions 7 and 8 also showed (tlc) two spots, the upper of which had the same R_f value as the lower spot of fraction 6. Fraction 9 (0.026 g.) had a small impurity (tlc), but was mainly one component. A low melting solid formed when fraction 9 was

treated with ether, and was purified by sublimation at 110° (0.05 mm). The 'sublimate' was a colourless oil which solidified into a white solid, and was identified as O-acetylfawcettiine 30 (i.r., m.s., tlc).

i.r. (Figure 4).

m.s. m/e: 349(M^+6), 289(17), 242(16), 234(100), 185(35), 174 (60), 146(20).

The methiodide derivative of fraction 9 (O-acetylfawcettiine) was obtained crystalline from acetone (0.006 g. methiodide from 0.0065 g. free base).

m.p.: 271-272°.

i.r.: (Figure 4a).

Fractions 10 through 13 were mixtures of at least two components (tlc) - O-acetylfawcettiine and a more polar component. Fractions 14, 15 and 16 (0.029 g.) contained as a minor constituent O-acetylfawcettiine but were made up mainly of another component, more polar than O-acetylfawcettiine. This component was crystallized from pentane-ether, and was identified as O-acetyldihydrolycopodine 31 (i.r., m.s., tlc).

m.p.: 94-96°.

i.r.: (Figure 5).

m.s. m/e: 291(M^+16), 234(100), 174(71), 146(20).

O-acetyldihydrolycopodine hydroperchlorate was prepared following the method described in the general experimental. It crystallized (0.003 g.) from acetone in an

ether atmosphere.

m.p.: 246-247°.

i.r.: (Figure 5a).

Fraction 17 showed one spot on tlc, and was not obtained crystalline. Together with similar material from other chromatograms (total, 0.026 g.) fraction 17 was identified as fawcettiine 32 (i.r., m.s., tlc, MeI).

i.r. ($CHCl_3$) : (Figure 6).

m.s. m/e: 307(M^+8), 234(85), 174(100), 146(44).

Fawcettiine methiodide was obtained as white crystals (0.002 g.) from methylene chloride-methanol (49:1).

m.p.: 293-294°.

i.r.: (Figure 6a).

Fractions 19 and 20 showed one spot each on tlc but only about 2 mg of this component was obtained. Fractions 18 and 21 through 30 did not contain appreciable basic material (tlc).

A further portion of this crude alkaloid was chromatographed over acid-washed alumina. The crude alkaloid (0.129 g.) was placed on a column of acid washed alumina (~ 4 g., 13 mm diameter) and eluted as in the previously described case. Fractions corresponding to the basic component of fractions 3, 4, and 5 described earlier, gave 0.007 g. of material. The compound was crystallized from pentane-ether and identified as lycopodine 1 (i.r., m.s., tlc).

m.p.: 115°.

i.r.: (Figure 3).

m.s. m/e: 247(M^+22), 190(100).

Lycopodine hydrochloride was prepared by treatment of lycopodine with methanolic hydrochloric acid. It was recrystallized from methanol in an ether atmosphere, and was identical with the crystal from the acetone solution of the crude alkaloids (m.p., i.r., m.s., tlc).

Material similar to the basic components of fractions 19 and 20 mentioned earlier, was obtained (0.015 g.) and combined with earlier fractions. This compound was not obtained crystalline.

i.r. ($CHCl_3$) : (Figure 7).

m.s. m/e: 305(M^+15), 246(23), 234(65), 174(100) 146(50).

A crystalline hydroperchlorate, m.p. 210-212°, was prepared. Its infrared spectrum is shown in Figure 7a.

L. Contiguum

The crude alkaloid (0.327 g., in chloroform) was placed on a column of aluminum oxide (BDH, ~ 20 g., diameter 23 mm) and eluted in 30, 3 ml portions (90 ml, eluent methylene chloride-methanol 49:1). The column was then further eluted in 10, 3 ml portions (30 ml, eluent methylene chloride-methanol 19:1).

Fractions 1 and 2 contained only solvent while fraction 3 showed two spots on tlc, only one of which was basic

(the lower spot). Fractions 4 and 5 showed one major spot and one minor one (upper spot). The major component had the same R_f value as the basic component of fraction 3. This component crystallizes from pentane ether and was identified as lycopodine 1 (i.r., m.s., tlc).

Fractions 6 and 7 showed two components, the upper (tlc) of which was lycopodine (R_f value). Fractions 8 and 9 also showed two spots, and the upper one had the same R_f value as the lower spot of fraction 7. Fractions 10 through 15 showed two spots, the upper of which had the same R_f value as the lower spot of fractions 8 and 9. Although fraction 16 also showed two spots, white needle-like crystals form from methyl acetate. This compound, along with similar material from other chromatograms (especially when eluent is methyl acetate), was identified as clavolonine 35 (i.r., m.s., tlc). A total of 26 mg. was isolated.

m.p.: 230°.

i.r.: (Figure 8).

m.s. m/e: 263(M^+5), 234(19), 190(100), 174(13), 162(6), 146(5).

Clavolonine methiodide (0.003 g.) was prepared by the method described in the general experimental (methanol as solvent). The crystals form in methanol.

m.p.: > 300°.

i.r.: (Figure 8a).

Fraction 17 was a mixture of clavolonine and a more

polar compound, but no crystals were obtained from this fraction. Fractions 18 through 22 showed one component with the same R_f value, so these fractions were combined (0.017 g.). Although this compound was not crystalline, it forms a precipitate in ether. This was identified as fawcettiine 32 (i.r., m.s., tlc). Fractions 23 through 30 did not contain any appreciable material. The chromatogram was next eluted with methylene chloride-methanol (49:1). Although fractions 37, 38, and 40 contained basic material, only fraction 38 gave an appreciable amount of material (0.006 g.). This material was similar to the unknown isolated from L. thyoides and was identified as such (i.r., m.s., tlc).

From other chromatograms, the material having the same R_f value as the lower spot in fractions 6 and 7 was isolated (0.010 g.). It was identified as O-acetylfawcettiine 30 (i.r., m.s., tlc).

L. Reflexum

The crude alkaloid was subjected to various thin-layer chromatograms (as listed in the results and discussions section of this thesis). Since no good separation was achieved, the crude alkaloid was acetylated as follows.

The crude alkaloid (0.100 g.) was dissolved in pyridine (3 ml), acetic anhydride (1.5 ml) was added, and the mixture left at room temperature overnight. The reagents were removed in vacuo and the residue chromato-

graphed over alumina.

The acetylated bases (total of 0.312 g.) were placed on a column of aluminum oxide (~ 20 g., 23 mm diameter) and eluted in 20, 5 ml fractions (100 ml, eluent chloroform-methanol 49:1). Fraction 1 showed one spot on tlc and gave 0.025 g. of a non-crystalline material when the solvent was evaporated.

uv $\lambda_{max}^{CH_3OH}$ nm: 305 (ε = 12,000).

i.r. ($CHCl_3$) cm^{-1}: 1735, (carbonyl), 1635 (C=C).

m.s. m/e: 527(1), 470(2), 347(8), 303(18), 288(14), 232(33), 215(17), 190(100), 43(87).

Fraction 2 showed two spots, the upper one has the same R_f value as the componeht of fraction 1. Fractions 3 and 4 showed one spot each with a little streaking above the spot in fraction 3, and below the spot in fraction 4. These spots had the same R_f value as the lower spot of fraction 2. Fractions 3 and 4 were combined and gave 0.042 g. of a non-crystalline material.

uv $\lambda_{max}^{CH_3OH}$ nm: 250 (ε = 14,000).

i.r. ($CHCl_3$) cm^{-1}: 3410(sharp,NH), 1740(shoulder, carbonyl), 1700 (shoulder, carbonyl), 1675 (carbonyl), 1630 (C=C).

Analysis. Calculated for $C_{17}H_{26}N_2O$: mw 274.2045.

Found: mw 274.2048 (h.r.m.s.).

m.s. m/e: 409(4), 367(3), 352(2), 274(23), 231(38), 217(100), 203(19), 189(26), 55(39), 44(40), 43(53), 42(45), 41(58).

Fractions 5 through 8 did not show any distinct spots. Fractions 9 and 10 showed one spot each. These spots have lower R_f values than the component of fractions 3 and 4. Fractions 9 and 10 were combined and after removal of solvent, 0.010 g. of a non-crystalline material was obtained.

uv λ_{max}^{MeOH} nm: 312 (ε = 5,400), 230 (ε = 7,600).

i.r. ($CHCl_3$) cm^{-1}: 3380 (NH), 1745 (shoulder, carbonyl) 1665, 1625, 1560 (characteristic of α-pyridones).

Analysis. Calculated for $C_{17}H_{24}N_2O$: mw 272.1889.

Found: mw 272.1887 (h.r.m.s.).

m.s. m/e: 512(2), 498(3), 484(25), 470(5), 456(7), 442(4), 291(6), 272(42), 255(16), 229(52), 215(100), 201(23), 187(22).

Fractions 11, 12 and 13 all showed two spots, the upper of which has the same R_f value as the component of fractions 9 and 10. The lower spot was almost at the origin. There was also a fair amount of material at the origin. Fractions 14 through 20 showed only material at the origin.

Attempted Formation of Hydroperchlorate Salts

The crude alkaloid (0.237 g.) was dissolved in methanol and perchloric acid in methanol added until the pH of the mixture was just acid (indicator paper, pH ~ 5). The mixture was allowed to stand at room temperature overnight. No crystals formed. Attempts to crystallize from methanol-acetone (mixture was insoluble in acetone), methanol in an acetone atmosphere and methanol in an ether atmosphere, all failed to give crystalline material.

Attempted Formation of Methiodide Salts

The crude alkaloid (0.300 g.) was dissolved in methanol and heated to reflux with excess methyl iodide for 30 min. The mixture was allowed to cool and the solution concentrated (steam bath). All attempts to induce crystallization were unsuccessful.

Attempted "Sublimation" of Crude Alkaloid

A few milligrams of the crude alkaloid were placed in a sublimation tube and the temperature gradually raised. No "sublimate" appeared at the cooled constriction even after heating to ~ 190° (0.6 mm).

III. SYNTHESIS

In Situ Preparation of α-Picolyllithium

Lithium metal (5.32 g., 0.76 moles) in small pieces, was introduced into a flask containing dry ethyl ether (45 ml) under nitrogen. 5 ml of a solution of bromobenzene in ether (0.38 moles in 80 ml ether) was added and the reaction mixture stirred vigorously to initiate the reaction. The remaining bromobenzene solution was added dropwise, and the mixture allowed to reflux for 2 hr.

α-Picoline (24.5 ml, 0.25 moles) was added dropwise to the reaction mixture, stirred continuously for 2 hr., then cooled to 0°C, to give α-picolyllithium.

2-(3-Butenyl)pyridine 47.

Allyl bromide (21.6 ml, 0.25 moles) dissolved in ether (38 ml) was added dropwise to the cooled flask containing α-picolyllithium. After addition was complete, the reaction mixture was allowed to warm up to room temperature and stirring was continued overnight. Distilled water (150 ml) was added dropwise, the aqueous layer separated and extracted three times with ether (80 ml). The combined organic phase was extracted several times with 6% HCl. The aqueous solution was basified with cold aqueous ammonia and extracted with chloroform. The chloroform extract was dried, concentrated, and the dark residue distilled under reduced pressure to give 2-(3-butenyl)pyridine (17.63 g., 53%) as a colourless liquid.

b.p. : 32.5° (0.4 mm).

i.r. cm^{-1}: 900, 985 (vinyl C-H), 1640 (C=C), 3030 (methine C-H).

n.m.r. δ : 2.7(m) ($-CH_2-CH_2-$), 5.0(m) ($H_2C=C$), 5.85(m) (C=C<u>H</u>), 7.30(m) (β and δ pyridine protons), 8.50(d) (α-pyridine proton).

Analysis. Calculated for $C_9H_{11}N$: mw 133.0890.

Found: mw 133.0880 (h.r.m.s.).

m.s. m/e: 133(M^+54), 132(100), 118(45), 116(35), 106(28), 93(20), 79(19), 65(18).

<u>2-(4-Hydroxylbutyl)pyridine 48</u>

2-(3-Butenyl)pyridine (6.65 g., 0.05 m) was dissolved in freshly distilled tetrahydrofuran (THF)(15 ml) at room temperature under nitrogen. 75 ml borane in THF (1 <u>M</u>) was added dropwise to the solution and stirring was continued for 2 hr. Excess borane was destroyed by addition of small pieces of ice, then 30 ml 3<u>N</u> aqueous NaOH was added. The mixture was cooled to 0°C, and 30% H_2O_2 (30 ml) was added dropwise. The mixture was stirred for 2 hr. then poured into water and extracted with ether. The ether solution was extracted with 6% HCl, the aqueous phase was basified (aqueous NaOH) and extracted with chloroform. The chloroform solution was dried, the solvent evaporated and the residue distilled under reduced pressure to give 2-(4-hydroxybutyl)pyridine <u>48</u> (6 g., 79.5%) as a colourless liquid.

b.p.: 102° (0.4 mm).

i.r. cm^{-1}: 3100 - 3600 (hydrogen bonded hydroxyl), 1050 (C-O), 750 (four CH_2 in a row).

n.m.r. δ: 1.74(m)($-CH_2CH_2-$ away from ring and OH), 2.82(t)($-CH_2-$ adjacent to ring), 3.66(t)($-CH_2-OH$), 4.50(s)(OH), 7.35(m)(ring protons), 8.50(d)(C-H adjacent to ring N).

Analysis. Calculated for $C_9H_{13}NO$: mw 151.0997.

Found: mw 151.0991 (h.r.m.s.).

m.s. m/e: 151(M^+3), 150(5), 134(12), 120(60), 106(67), 93(100).

Tetrahydroquinolizinium Bromide (50)

2-(4-Hydroxybutyl)pyridine (2.986 g., 0.0198 m) was added to a mixture of 98% H_2SO_4 (2.2 g.) and 48% HBr (6.7 g., 0.0396 m). The solution was heated under reflux for 6 1/2 hrs., then poured into water, basified with aqueous NaOH and extracted with chloroform. The chloroform solution was dried, and the solvent evaporated, to give a crystalline residue which was identified as tetrahydroquinolizinium bromide 50. Pure tetrahydroquinolizinium bromide (3.545 g., 83.7%) was obtained after thorough washing of the residue with ether and acetone.

m.p.: 237-240°.

i.r. cm^{-1}: 760 (four CH_2 in a row).

n.m.r. δ: 2.2(m)(β + α $-CH_2-$), 3.45(t)($-\beta'$ CH_2), 4.98(t)(α - CH_2 -), 8.50(m)(β + α pyridine protons), 9.62(d)(α-pyridine proton).

Quinolizidine (43)

Platinum oxide (0.04 g.) in methanol was added to a solution of tetrahydroquinolizinium bromide (0.5 g.) in methanol. The mixture was hydrogenated (50 psi) overnight, filtered and the solvent evaporated. Recrystallization of the crystalline residue from acetone gave quinolizidine hydrobromide 51 (0.473 g., 92%).

m.p.: 292-293°.

i.r. cm^{-1}: 2500 - 2700 (Bohlmann bands and $-\overset{\oplus}{N}H$).

n.m.r.: complex, with peaks between 51.65 and 53.6.

Analysis. Calculated for $C_9H_{18}NBr$: C 49.10%; Br 36.30%; H 8.24%; N 6.36%.

Found: C 49.29%; Br 36.29%; H 8.15%; N 6.27%.

Liberation of Quinolizidine 43

A hydroxide loaded ion-exchange resin column was prepared as follows. Amberlite ion-exchange resin (5 g., IRA - 400 C.P. Mallinckrodt, $RN(CH_3)_3^{\oplus}Cl^{\ominus}$, medium porosity) was thoroughly washed with 5% aqueous NaOH until no more chloride ions were detected ($AgNO_3$). The column was washed with distilled water until the eluant was neutral to indic-

ator paper. Excess water was drained, a solution of quinolizidine hydrobomide in water was applied to the column which was then eluted with water. The eluant was extracted with chloroform. The chloroform extract was dried and the solvent evaporated. Quinolizidine was isolated as an oil.

i.r. cm^{-1}: 2670, 2740, 2780 (Bohlmann bands), 1110 (C-N).

n.m.r.: overlapping peaks between δ 1 and δ 3

Analysis. Calculated for $C_9H_{16}N$: mw 138.1283.

Found: mw 138.1278 (h.r.m.s.).

m.s. m/e: 139(M^+50), 138(100), 110(49), 97(98), 83(95).

Quinolizidine N-oxide (42).

A solution of 99% meta-chloroperbenzoic acid (0.436 g.) in chloroform was added to a cold (0°) solution of quinolizidine (0.294 g.) in chloroform. The mixture was stirred at room temperature overnight, then chromatographed over aluminum oxide. Elution with chloroform removed all unreacted quinolizidine. Quinolizidine N-oxide (0.262 g., 80%) was obtained by elution with chloroform-methanol (3:1), as an oil which developed a few needle-like crystals on standing at room temperature for days.

i.r. cm^{-1}: 950($\overset{\oplus}{N} - \overset{\ominus}{O}$), 1130(C-N).

^{13}C n.m.r.: 20.3918(t), 23.1430(t), 26.9736(t) 68.4042(t), 71.6410(d).

Analysis. Calculated for $C_9H_{17}NO$: mw 155.1310.

Found: mw 155.1306 (h.r.m.s.).

m.s. m/e: 155(M^+35), 138(50), 100(100), 83(30).

Lycopodine N-oxide 40

Using the procedure described above for quinolizidine, lycopodine was transformed into lycopodine N-oxide in 81.2% yield.

m.p.: 234-236°.

i.r. cm^{-1}: 1710 (C=O), 950 ($\overset{\oplus}{N} - \overset{\ominus}{O}$).

Analysis. Calculated for $C_{16}H_{25}NO_2$: mw 263.1885.

Found: mw 263.1896 (h.r.m.s.).

m.s. m/e: 263(M^+39), 245(41), 218(43), 190(100).

Attempted Pyrolysis of Lycopodine N-oxide 40

i) in air:

A few milligrams of lycopodine N-oxide were placed in a small tube within a sublimation tube. The sublimation tube was connected to a vacuum line, and heated in a heating block. The area of the sublimation tube just outside of the heating block was cooled with a wet cloth. Heating was controlled by a "powerstat" variac. The temperature was gradually raised until some material began to "sublime". At 150° (1.4 mm) a yellow substance began to appear at the cooled portion of the tube. The temperature was held at 150° overnight, still only the yellow substance "sublimed". This substance was characterized and

shown to be lycopodine (i.r., m.s., tlc). The sample was then heated to 170° (1.0 mm), still only more yellow material "sublimed". At 193° (1.0 mm), the sublimate was still lycopodine, but the residue began to darken. The result was the same at 205° (0.4 mm).

ii) under nitrogen:

A few milligrams of lycopodine N-oxide were placed in a 3-necked flask that had been flushed with nitrogen. The flask was connected to a vacuum line *via* a condenser and heated to 215°-225° (1.2 mm). Initially (215°) the yellow substance was again the product, condensing on the cooler parts of the flask. Later (220°) a brown dust also began to collect on the cooler parts of the flask. The yellow material was identified as lycopodine (tlc) while the brown dust was identified as lycopodine N-oxide (tlc).

Reaction of Lycopodine N-oxide With Potassium Hydroxide.

i) in ethylene glycol:

Lycopodine N-oxide (0.116 g.) was dissolved in freshly distilled (68°, 1.2 mm) ethylene glycol under a slow stream of nitrogen. A solution of KOH (0.101 g.) in ethylene glycol (with a few drops of water to aid solubility) was added and the mixture heated under reflux at 200°C for 3 1/2 hrs. After cooling to room temperature the reaction

mixture was poured into water and extracted with chloroform. The chloroform extract was dried, evaporated to dryness, then subjected to acid-base extraction, to give lycopodine (0.088 g.).

i.r. ($CHCl_3$) cm^{-1}: 1700 (C=O).

m.s. m/e: 247(M^+), 190(100).

ii) in DMSO:

Lycopodine was isolated as the only reaction product, following the procedure described above (i) except that DMSO was used as solvent.

iii) in the absence of KOH with ethylene glycol as solvent:

Again lycopodine was the only reaction product isolated, using the same conditions as (i) but omitting the KOH.

Dihydrolycopodine

i) from lycopodine and sodium borohydride in methanol:

Lycopodine (0.4 g.) was dissolved in methanol (5 mls), Na_2CO_3 (0.02 g.) and $NaBH_4$ (0.1 g.) were added, and the mixture stirred at room temperature overnight. The solvent was removed, water added and the aqueous solution extracted with ether. The ether solution was dried and the solvent evaporated to give a crystalline product

(0.397 g.). Chromatographic separation of the product gave 0.273 g. dihydrolycopodine.

ii) in ethanol:

Lycopodine (0.4 g.) was dissolved in ethanol, $NaBH_4$ (0.1 g.) was added and the mixture was stirred overnight. More $NaBH_4$ (0.05 g.) was added and the mixture heated under reflux for 2 hr. The solvent was removed, aqueous Na_2CO_3 (50 ml) was added and the aqueous solution extracted with ether. The ether solution was dried and the solvent evaporated to give dihydrolycopodine m.p. 169°, (0.388 g., 96.5%), as the only product.

Dihydrolycopodine Methiodide 56

Following the procedure described in the general experimental, dihydrolycopodine methiodide was prepared in 86% yield as white crystals.

m.p.: 287-289°.

i.r. cm^{-1}: 3330 (hydrogen bonded OH), no carbonyl absorption.

Attempted Hofmann - Elimination of Dihydrolycopodine Methiodide.

Dihydrolycopodine methiodide (0.159 g.) was dissolved in freshly distilled anhydrous tert-butyl alcohol (40 ml). 1M $K^{\oplus}\,{}^{\ominus}O^{t}Bu$ in $^{t}BuOH$ (2 ml, ~ 4 eq.) was added and the reaction mixture heated under reflux overnight. The solvent was removed, water added, and the aqueous solution

extracted with ether. The ether solution was dried and evaporated to dryness. Separation of the components by preparative tlc on silica gel in chloroform-methanol (94:6) gave 0.024 g. of a basic compound, identified as compound 27 by comparison of spectroscopic data with that reported by MacLean.[19]

i.r. ($CHCl_3$): Figure 9.

m.s. m/e: 261(M^+25), 233(23), 218(15), 70(22), 57(100).

Attempted Hofmann - Elimination in DMSO.

$K^{\oplus}\ ^{\ominus}O^tBu$ (0.235 g., ~ 4 eq.) was dissolved in dry freshly distilled DMSO (25 ml) dihydrolycopodine methiodide (0.200 g.) was added and the mixture was stirred overnight at room temperature. The solvent was removed, water added and the aqueous solution was extracted with ether. The ether extract was dried and removal of solvent gave 0.060 g. of crude product. Separation by preparative tlc afforded a nitrogenous base whose mass spectrum shows a base peak at m/e 58. This compound is described in more detail below.

Hofmann - Elimination in DMSO Under Nitrogen.

Freshly sublimed $K^{\oplus}\ ^{\ominus}O^tBu$ (210°, 1 mm, 0.230 g.) was dissolved in freshly distilled anhydrous DMSO (50 ml) under nitrogen. Dihydrolycopodine methiodide (0.200 g.) was added and the mixture was stirred overnight at room temp-

erature under a slow stream of nitrogen. The DMSO was removed *in vacuo*, water added and the aqueous solution was extracted with ether. Evaporation of the dried ether solution, gave 0.030 g. of crude product. A nitrogenous base Q was isolated by preparative tlc. This same base Q (tlc) was isolated when the reaction was carried out at 80°C. At 80° the yield of crude product was increased (0.270 g. dihydrolycopodine methiodide gave 0.146 g. crude product, from which 0.063 g. of base Q was isolated as an oil by preparative tlc). This oily product partially solidified on standing.

i.r. (neat) cm^{-1}: 960, 972 (mono substituted double bond), 1365 ($N-CH_3$), 1382 ($C-CH_3$), 1585 (C=C), 2760 ($>N-CH_3$), 3060 (olefinic methine hydrogens).

i.r. ($CHCl_3$): (Figure 10).

m.s. m/e: 273(37), 258(12), 244(8), 230(12), 218(11), 216(10), 192(10), 164(16), 150(40), 91(31), 84(71), 70(48), 58(100).

Chemical ionization (NH_3) : ($M + H^+$) 274. A small peak was also observed at m/e 288.

High resolution mass spectral data:

Formula	Calculated	Found
$C_{19}H_{31}N$	273.2457	273.2454
$C_{18}H_{28}N$	258.2222	258.2223
$C_{15}H_{24}N$	218.1909	218.1914
$C_{15}H_{22}N$	216.1752	216.1753
$C_{10}H_{16}N$	150.1283	150.1284
$C_5H_{10}N$	84.0813	84.0811

n.m.r.: (Figure 11).

The methiodide of this product crystallizes from methanol-ethylacetate (1:2) in an ether atmosphere.

m.p.: 190-191°.

i.r.: (Figure 10a).

n.m.r.: (Figure 11a).

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Figure 1
L. thyoides

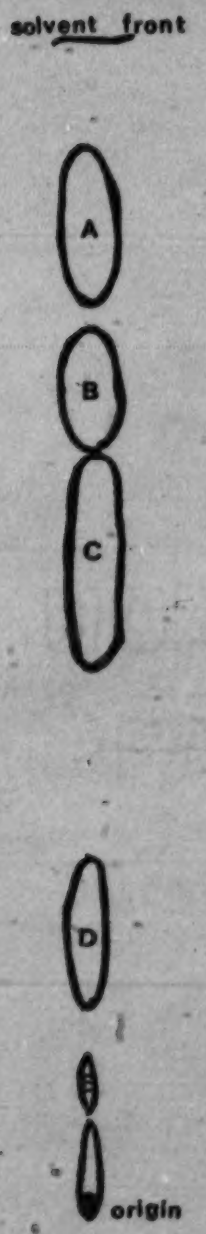
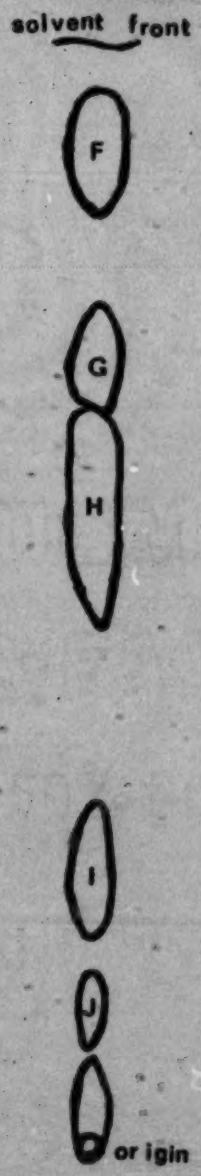


Figure 2
L. contiguum



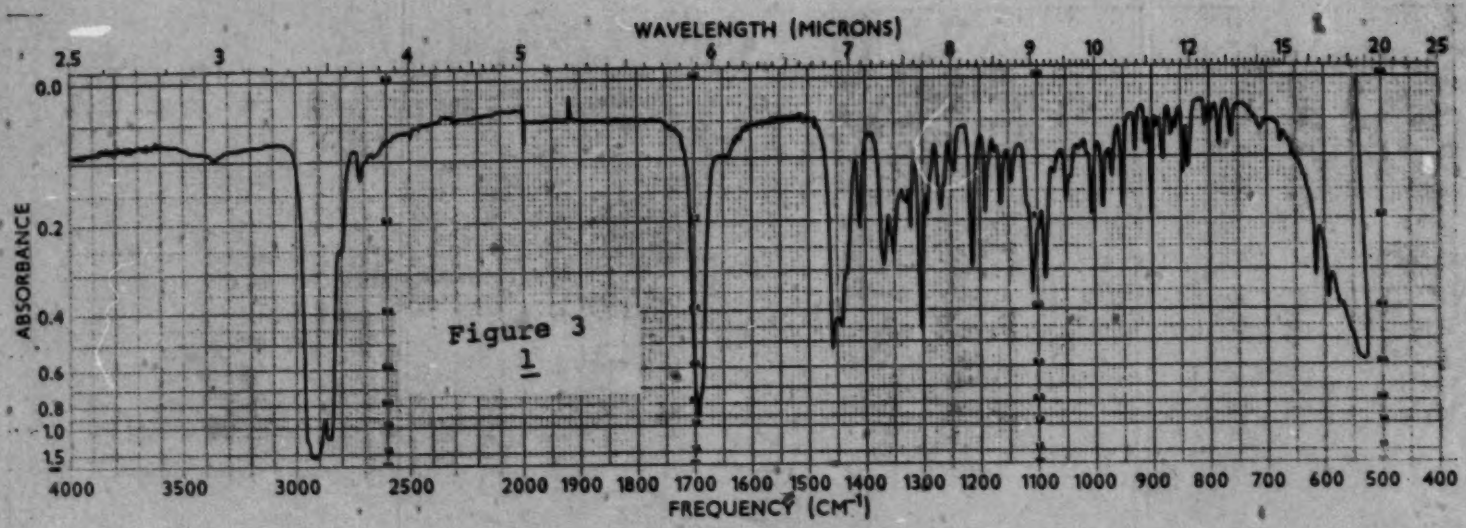


Figure 3
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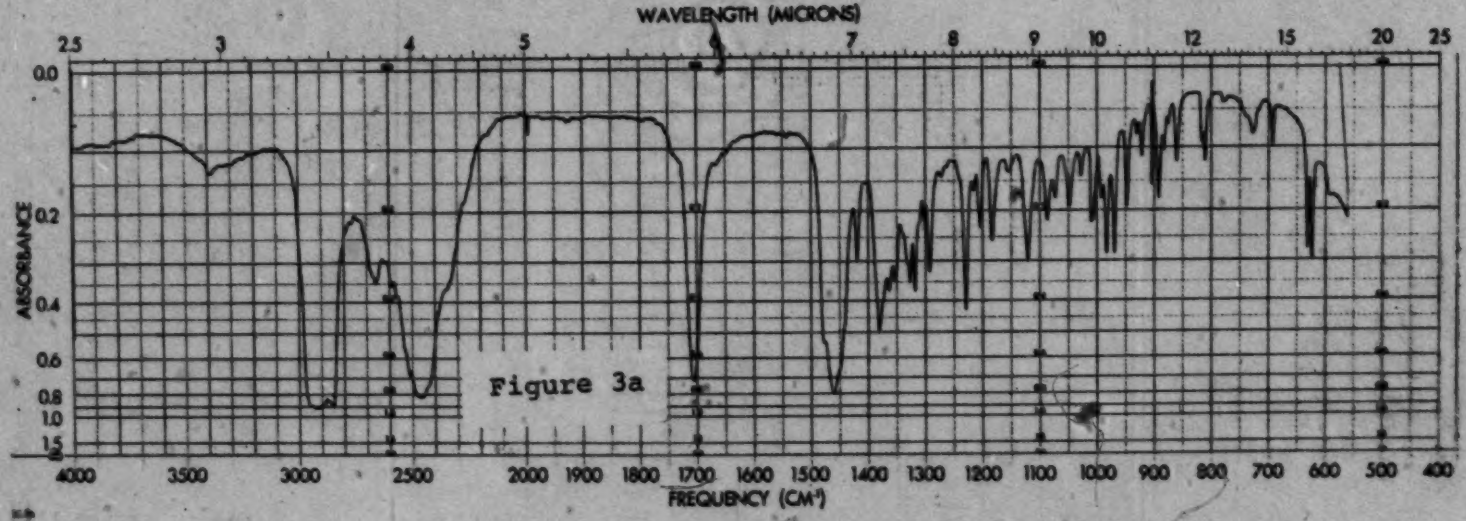

Figure 3a

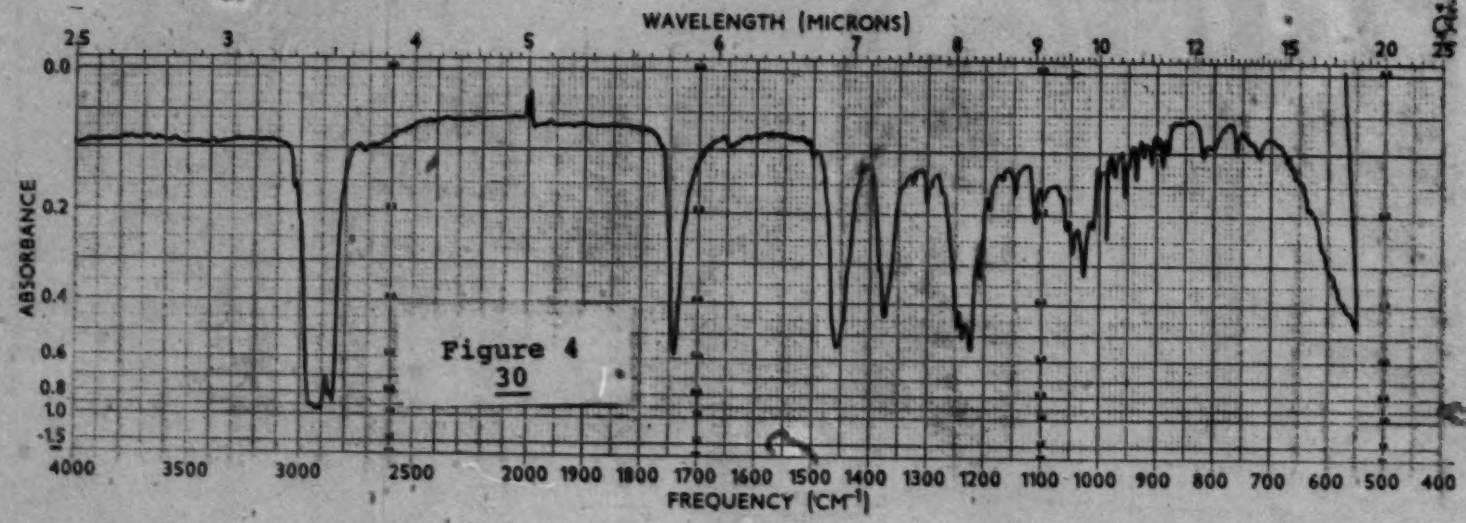

Figure 4

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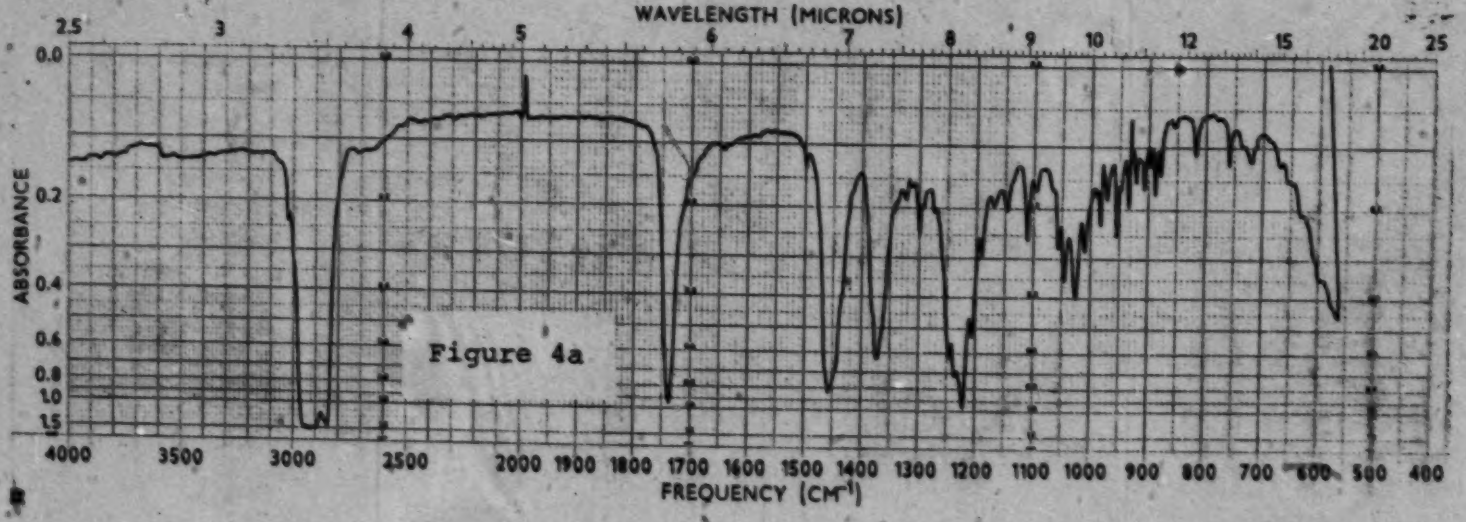

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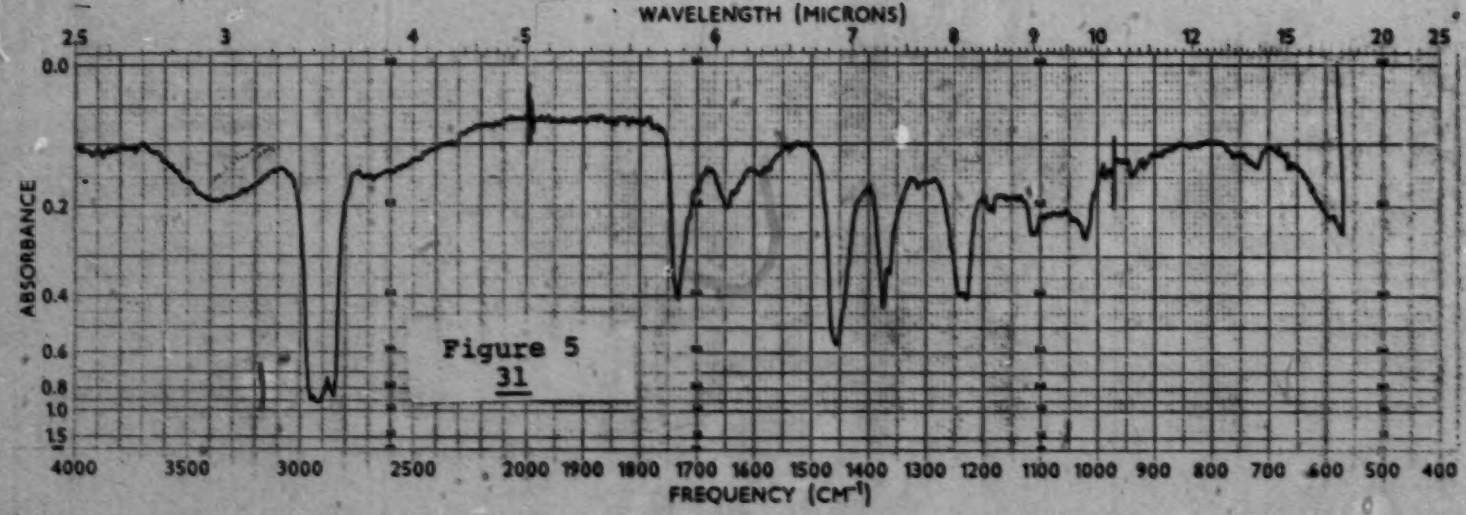

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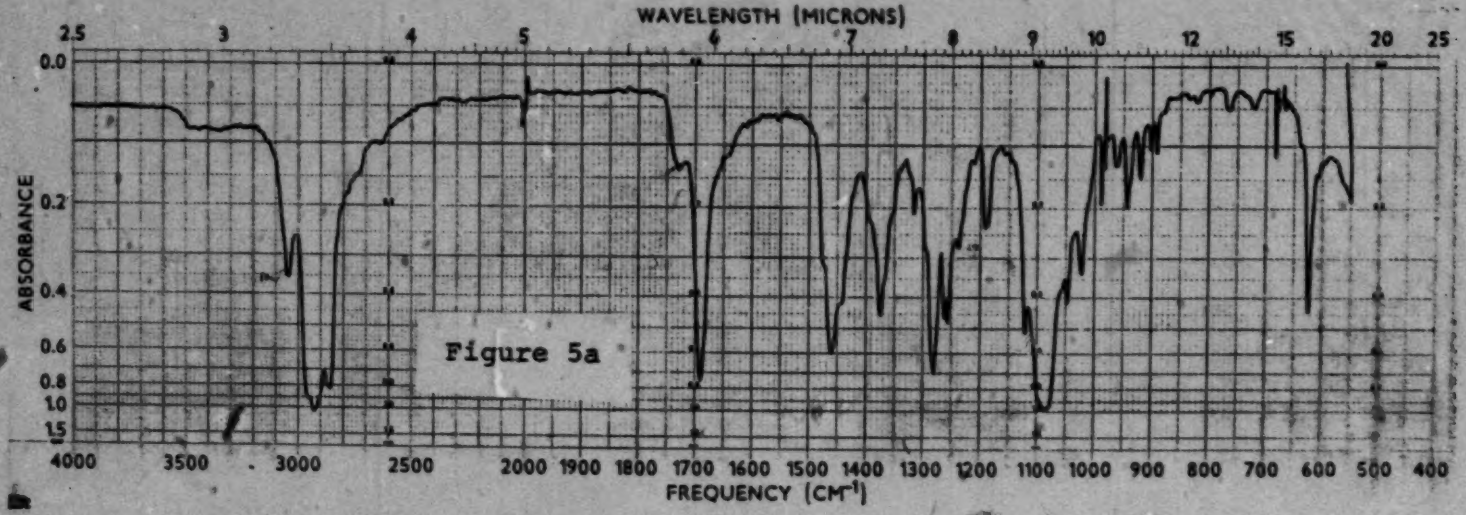
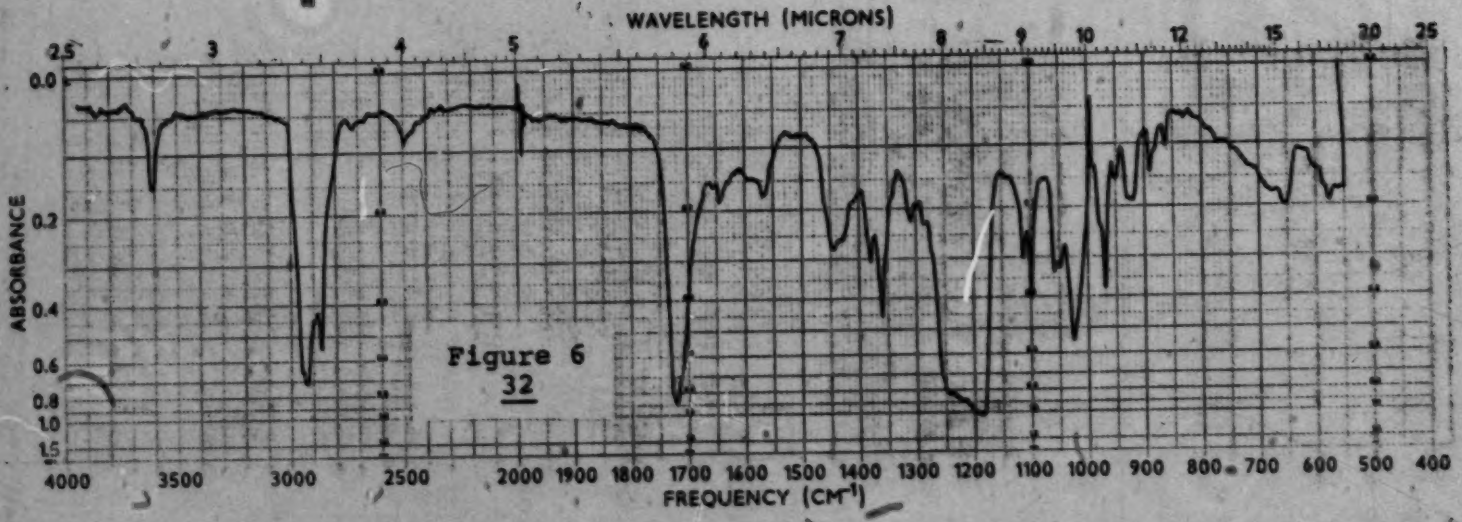


Figure 5a



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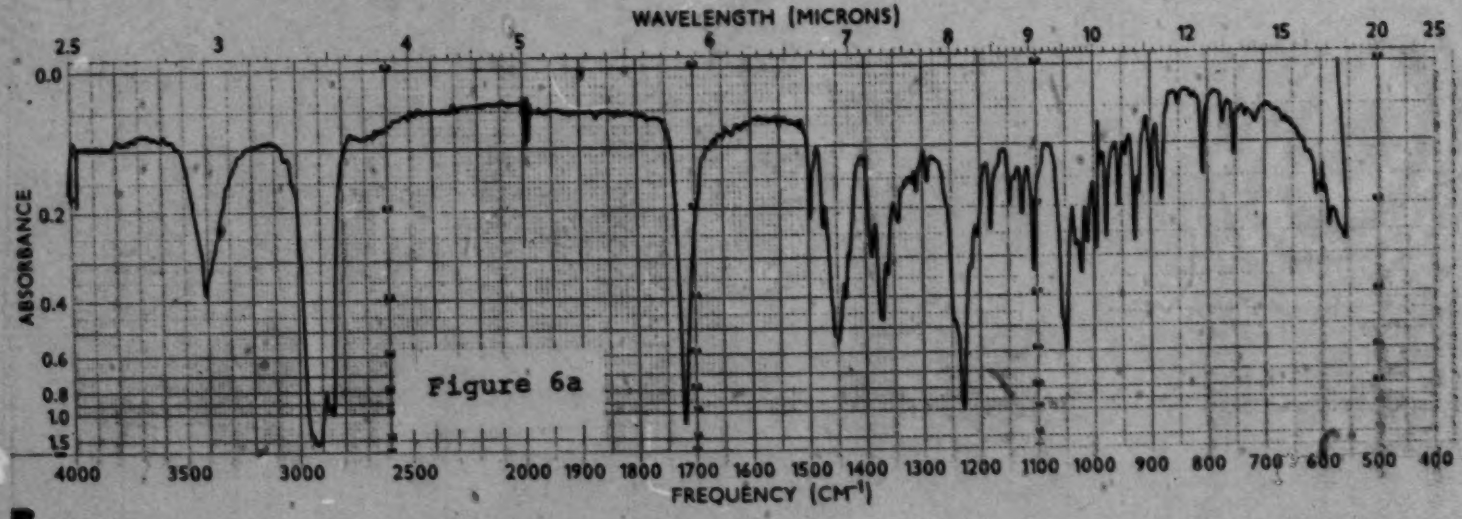
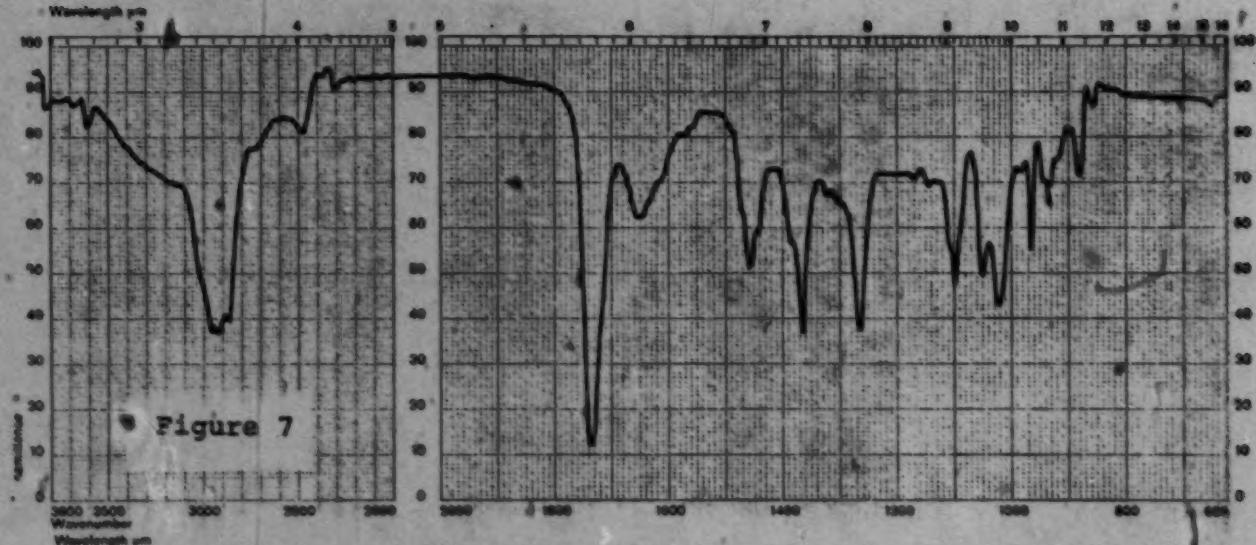


Figure 6a



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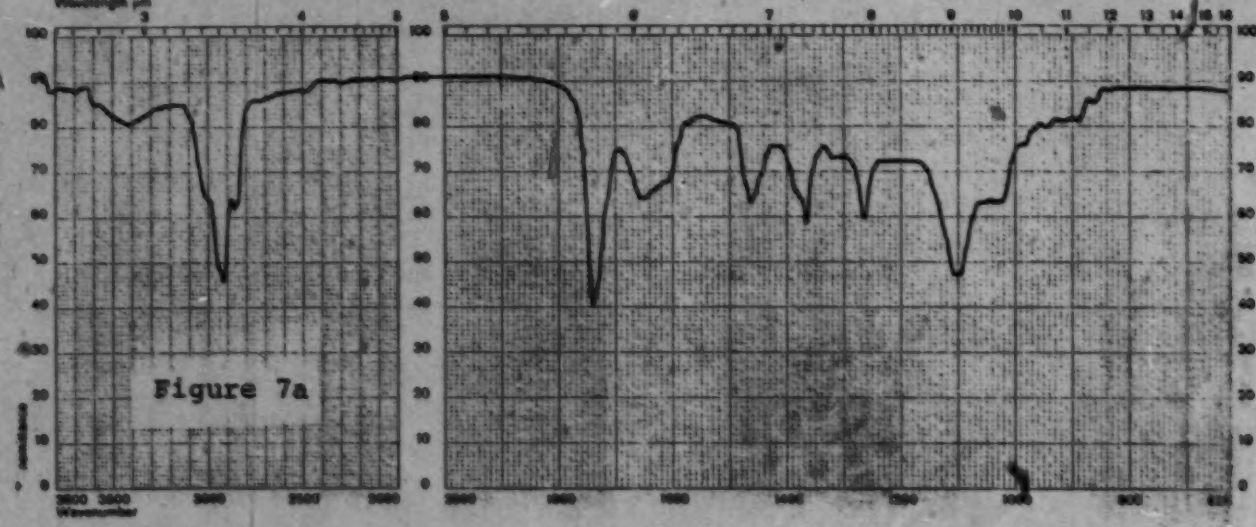

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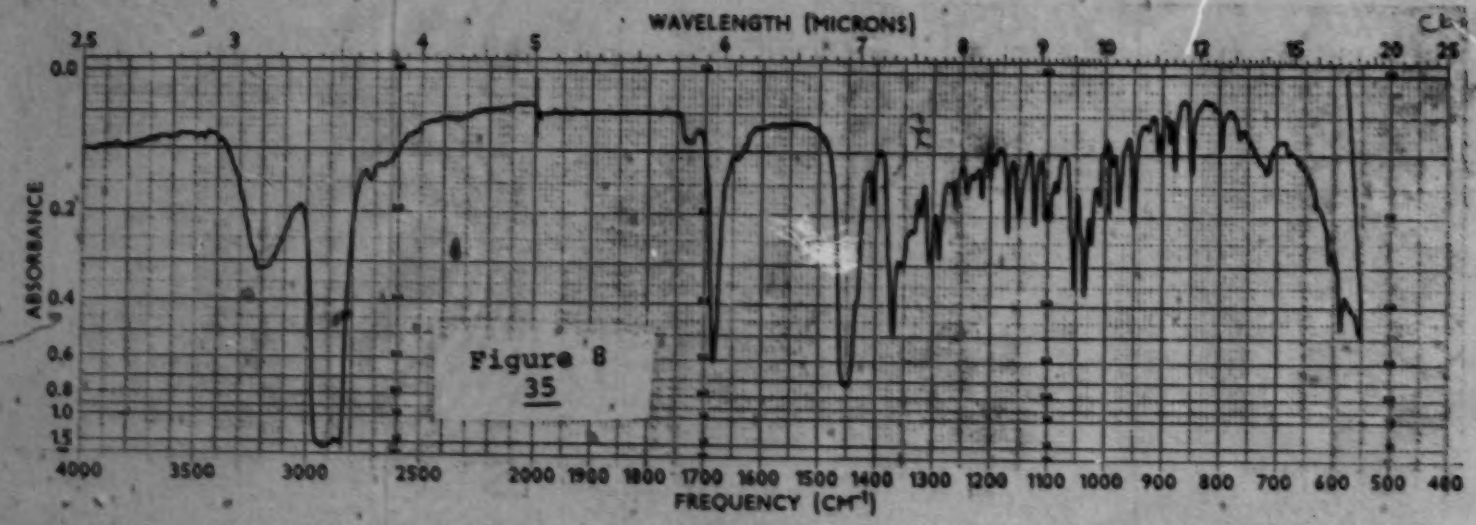
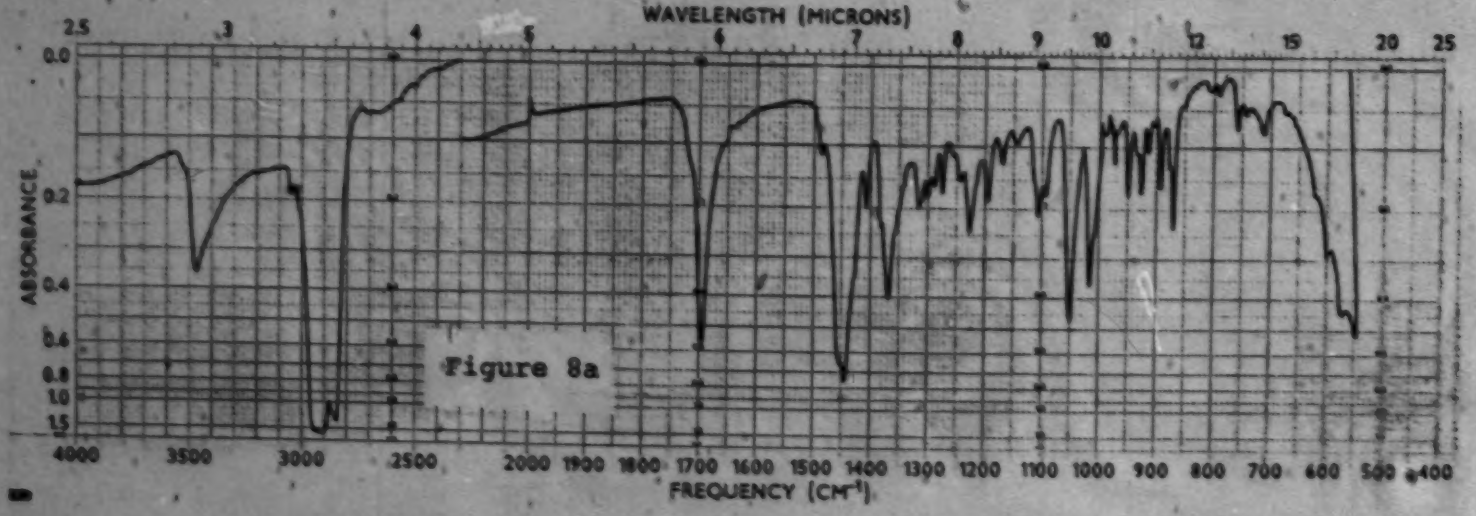


Figure 8

35



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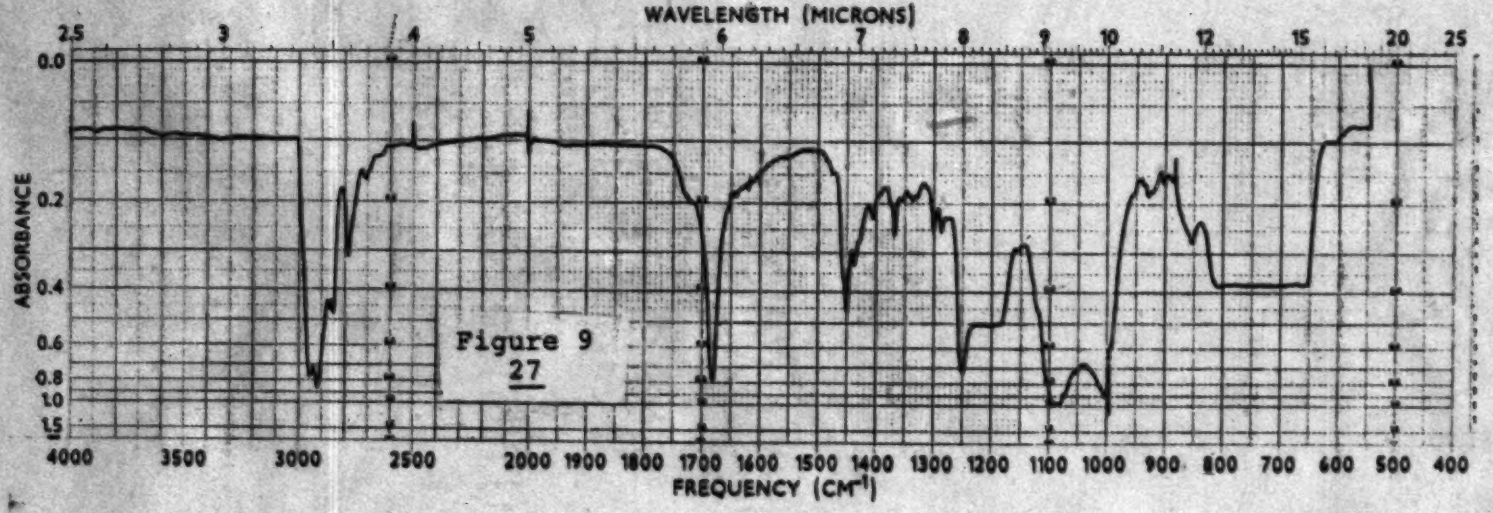
WAVELENGTH (MICRONS)
2.5
3
4
5
6
7
8
9
10
12
15
20
25
ABSORBANCE
0.0
0.2
0.4
0.6
0.8
1.0
1.5
4000
3500
3000
2500
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
FREQUENCY (CM⁻¹)

Figure 9
27

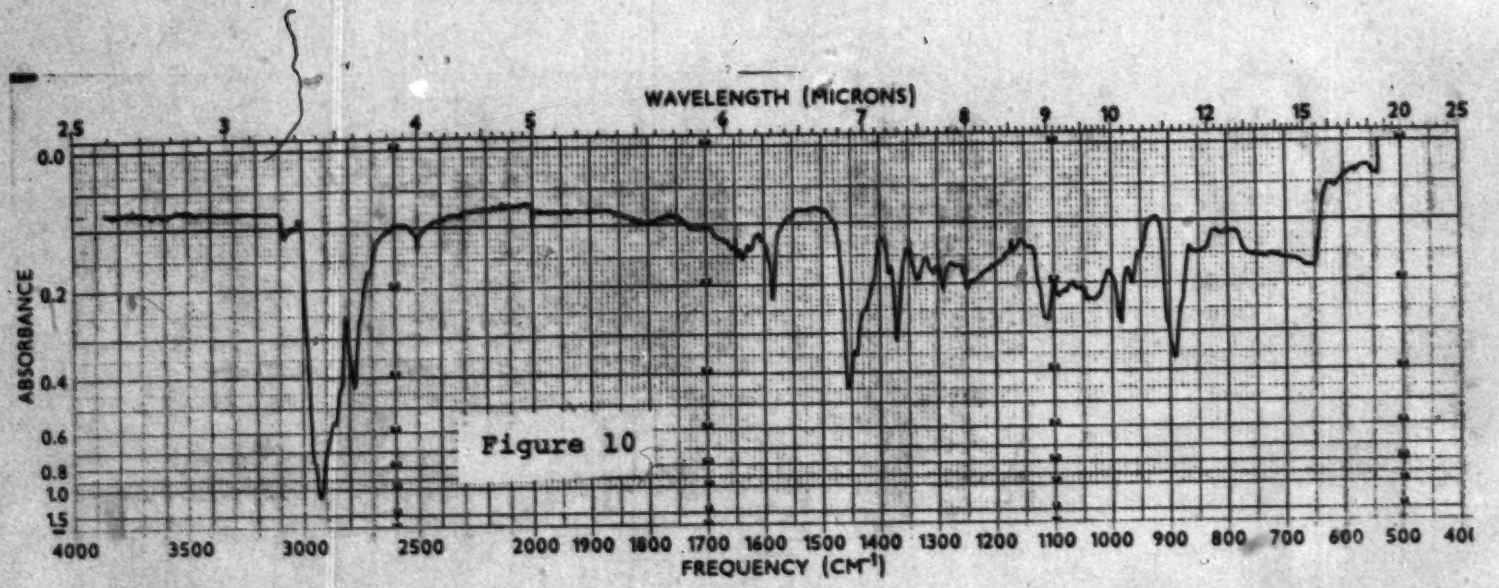

Figure 10

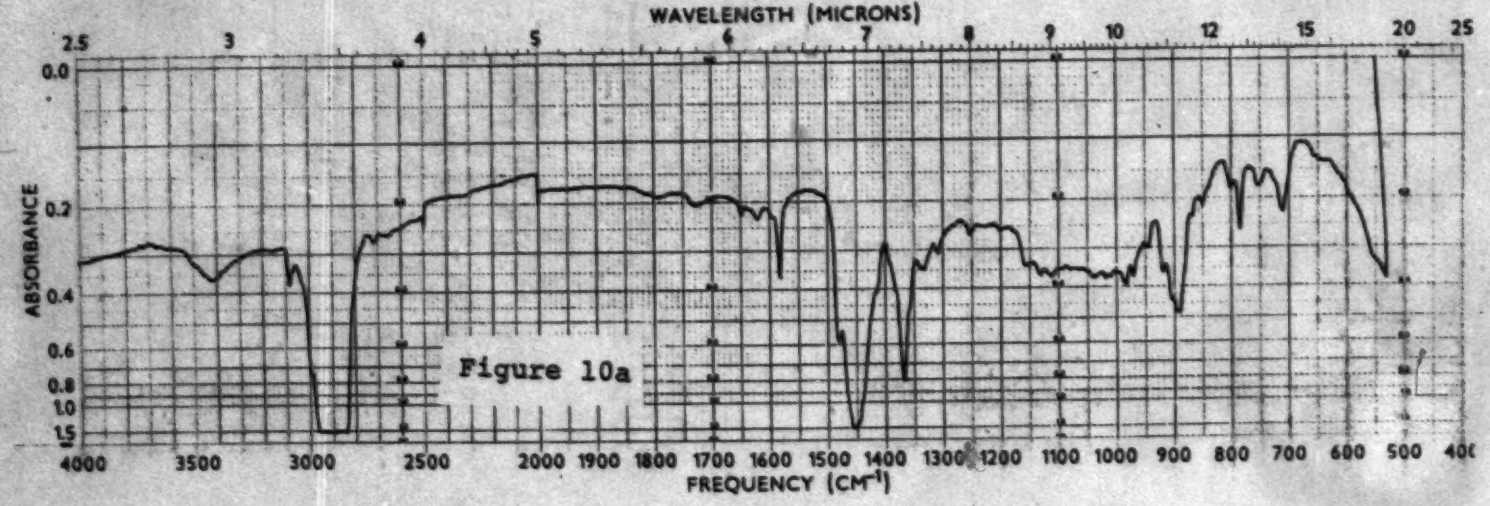

Figure 10a

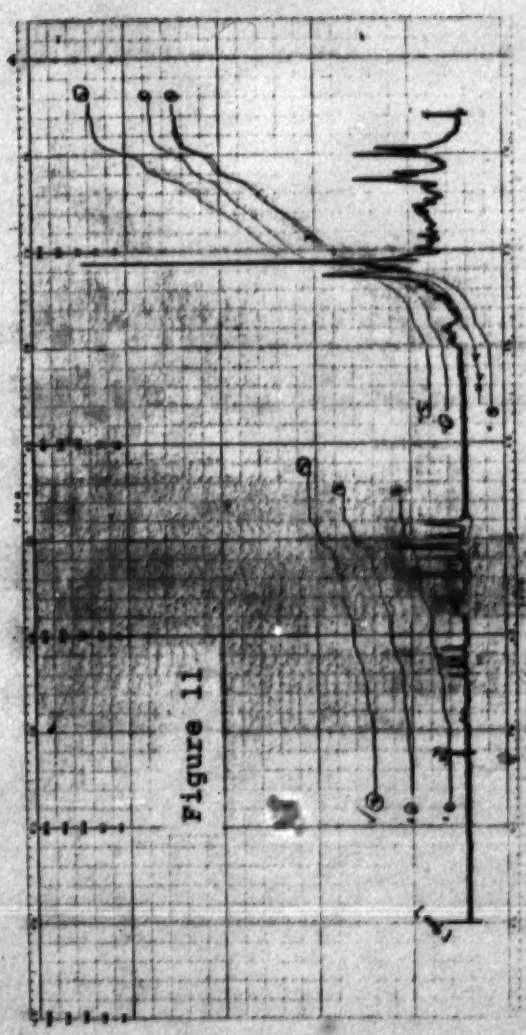

Figure 11

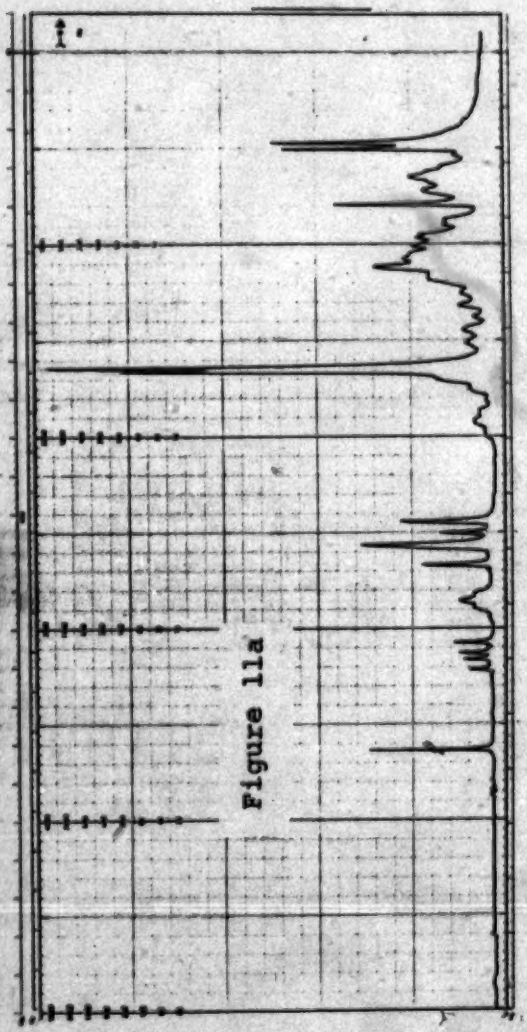

Figure 11a

REFERENCES

1. D.B. MacLean, The Alkaloids X, edited by R.H. Manske p. 305.
2. W.A. Ayer in MTP International Review of Science, Organic Chemistry, 9, 1 (1973).
3. K. Wiesner, Fortsch. Chem. Org. Naturstoffe, 20, 271 (1962).
4. W.A. Ayer, Y. Fukazawa, P. Singer and B. Altenkirk, Tetrahedron Lett., 50, 5045 (1973).
5. G. Stork, R.A. Kretchmer and R.H. Schlessinger, J. Amer. Chem. Soc., 90, 1647 (1968).
6. W.A. Ayer, W.R. Bowman, T.C. Joseph and P. Smith, J. Amer. Chem. Soc., 90, 1648 (1968).
7. K. Wiesner and L. Poon, Tetrahedron Lett., 4937 (1967).
8. K. Wiesner, L. Poon, I. Jirkovsky and M. Fishman, Can. J. Chem., 47, 433 (1969).
9. H. Harayama, M. Ohtani, M. Oki and Y. Inubushi, pre-print paper, October (1974).
10. W.A. Ayer and K. Piers, Can. J. Chem., 45, 451 (1967).
11. F.A.L. Anet and M.V. Rao, Tetrahedron Lett., #20, 9 (1960).
12. H. Conroy, Tetrahedron Lett., #10, 34 (1960).
13. R.N. Gupta, M. Castillo, D.B. MacLean, I.D. Spenser and J.T. Wrobel, J. Amer. Chem. Soc., 90, 1360 (1968).
14. R.N. Gupta, M. Castillo, D.B. MacLean and I.D. Spenser, Can. J. Chem., 48, 1893 (1970).

15. E. Leete, J. Amer. Chem. Soc., 86, 2509 (1964).

16. M. Castillo, R.N. Gupta, Y.K. Ho, D.B. MacLean, I.D. Spenser, Can. J. Chem., 48, 2911 (1970).

17. J.C. Brackman, R.N. Gupta, D.B. MacLean and I.D. Spenser, 4th Natural Products Symposium, University of the West Indies, Abstracts of Papers, 14, (Kingston), (1972).

18. H. Dugas, M.E. Hazenberg, Z. Valenta and K. Wiesner, Tetrahedron Lett., 4931 (1967).

19. N. Chin-You, D.B. MacLean, A. Prakash, C. Calvo, Can. J. Chem., 49, 3240 (1971).

20. W.A. Ayer et al., Can. J. Chem., 45, 433 (1967).

21. W.A. Ayer, A.N. Hogg and A.C. Soper, Can. J. Chem., 42, 949 (1964).

22. A.C. Cope and E.R. Trumbull, Org. Reactions, 11, 317 (1960).

23. O. Touster, Org. Reactions, 7, Chap. 6 (1953).

24. A.C. Cope and N.A. LaBel, J. Amer. Chem. Soc., 82, 4656 (1960).

25. V. Boekelheide and J.P. Lodge, J. Amer. Chem. Soc., 73, 3681 (1951).

26. K. Winterfeld and W. Dunwald, Naturewissenschaften, 43, 517 (1956).

27. V. Boekelheide and W.G. Gall, J. Amer. Chem. Soc., 76, 1832 (1954).

28. E.E. Glover and G. Jones, J. Chem. Soc., 3021 (1958).

29. A. Richards and T.S. Stevens, J. Chem. Soc., 3067 (1958).

30. J.C. Craig and K.K. Purushothaman, J. Org. Chem., 35, 1721 (1970).

31. N.N. Schwartz and J.H. Blumbergs, J. Org. Chem., 29, 1976 (1964).

32. W.A. Harrison and D.B. MacLean, Can. J. Chem., 39, 2086 (1961).